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## ERYTHROLEUKOSIS AND THE ANEMIAS OF THE FOWL\*

J. FURTH, M.D.

WITH THE ASSISTANCE OF RUTH KLINGELHOFER, B.S., AND CHARLES BREEDIS  
PHILADELPHIA

In the course of his studies on the transmissible leukemia of fowls Ellermann<sup>1</sup> observed the frequent occurrence of anemia. On microscopic examination of various organs, particularly of the liver, spleen and bone marrow, many of the anemic birds showed an engorgement of the capillaries with lymphoid cells. On the basis of this characteristic, Ellermann considered the condition, to which he at first gave the name intravascular lymphoid leukosis, a distinct disease. His later studies on the nature of these lymphoid cells led him to surmise that they were progenitors of erythrocytes, and he accordingly changed the name of this pathologic condition to erythroleukosis.

Many of the birds inoculated by Ellermann with the virus of leukemia showed anemia during life, but on microscopic examination of the organs did not reveal the characters of erythroleukosis. These cases are designated by him as simple anemia. Simple anemia and erythroleukosis, according to Ellermann, are varieties of the same disease with all possible transitional forms existing between them.

Most of the investigators working with the leukemia of fowls have observed the occurrence of anemia, but none of them, so far as I am aware, has described cases of erythroleukosis. Data on the spontaneous occurrence of erythroleukosis have been wanting. It is not known whether chronic severe anemia in the bird would resemble erythroleukosis. Cases of erythroleukosis were probably observed by Andersen and Bang<sup>2</sup> and by McGowan,<sup>3</sup> but were not separated from myeloid leukosis.

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\* Submitted for publication, Nov. 21, 1930.

\* From the Henry Phipps Institute, University of Pennsylvania.

\* This investigation has been supported by the Fund for the Study of Leucemia and Related Diseases.

1. Ellermann, V.: *The Leucosis of Fowls and Leucemia Problems*, London, Gyldenal, 1927; *Virchows Arch. f. path. Anat.* **228**:247, 1920.

2. Andersen, C. W., and Bang, O.: *Festkrift til B Bang*, Copenhagen, 1928, p. 353.

3. McGowan, J. P.: *Pernicious Anemia, Leucemia and Aplastic Anemia*, New York, Paul B. Hoeber, 1927.

Recently Bedson and Knight<sup>4</sup> described in the fowl a severe spontaneous anemia, which is apparently not erythroleukosis and when transmitted to normal fowls is not capable of producing either progressive anemia or erythroleukosis.

Erythroleukosis offers an excellent opportunity for the study of the lymphoid precursors of erythrocytes.

#### ERYTHROPOIESIS IN THE BIRD

The bone marrow is the only organ of the adult bird in which red cells are formed. The structure and function of the avian marrow, although comparatively simple, are not wholly understood. The marrow is formed by a network of blood vessels, lined with a single layer of endothelium, some dilated, others collapsed. The tissues between the vessels, commonly called trabeculae or parenchyma, contain fat or some homogeneous "gelatinous" material.

Bizzozero and Torre<sup>5</sup> recognized that erythropoiesis and granulocytopoiesis are sharply separated in the marrow of the bird, the former occurring within the venous capillaries, the latter extravascularly in the parenchyma of the marrow. Their observations have been repeatedly confirmed (Van der Stricht,<sup>6</sup> Sabin<sup>7</sup>), and the extensive material presented in this report also substantiates this point of view. Indeed it would seem that when under pathologic conditions erythrocytogenesis occurs outside of the marrow it is in most, if not in all, instances an intravascular process.

The circulatory system of the marrow is thus intimately connected with the formation of red cells. Sabin,<sup>7</sup> in her recent review on the bone marrow, summarized the available evidence pointing to a closed circulation as recognized by Bizzozero. Attention is called to the significant difference between dilated and collapsed vessels, sluggish flow favoring erythropoiesis (Bizzozero), presumably through low tension of oxygen. Maximow,<sup>8</sup> following Mollier's<sup>9</sup> idea, conceived the erythropoietic sinusoids of the marrow as vessels with fenestrated walls formed by histiocytes with embryonic potencies, capable of constriction and dilation. The former view (Sabin<sup>7</sup>) explains the delivery of the newly formed red blood cells by the opening up of the collapsed capillaries and explains the delivery of the white cells by active migra-

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4. Bedson, S. P., and Knight, E.: *J. Path. & Bact.* **27**:239, 1924.

5. Bizzozero, G.: *Arch. f. mikr. Anat.* **35**:424, 1890.

6. Van der Stricht, O.: *Arch. de biol.* **12**:199, 1892.

7. Sabin, F. R.: *Physiol. Rev.* **8**:191, 1928.

8. Maximow, A.: *Bindegewebe und blutbildende Gewebe*, in Möllendorff: *Handbuch der mikroskopischen Anatomie*, Berlin, Julius Springer, 1927, vol. 2.

9. Mollier, S.: *Arch. f. mikr. Anat.* **76**:608, 1910-1911; **74**:474, 1909.

tion; the latter emphasizes contraction of the histiocytic cells and the fenestrae of the capillaries in explaining the entrance of nonmotile cells into the circulation.

The immediate precursors of erythrocytes are erythroblasts (Bizzozero,<sup>5</sup> Denys<sup>10</sup>). These in the bird are characterized by being round. Their cytoplasm has a peculiar color, given to it by the varying amount of hemoglobin and by the so-called basophil substance that they contain. The more mature forms contain relatively much hemoglobin and are only slightly basophil (erythroblasts II, Sabin<sup>7</sup>). They will be designated in this report as polychrome erythroblasts, a term introduced by Gabritschewski. The younger forms of the erythroblasts contain only traces of hemoglobin and much "basophil substance" (erythroblasts I, Sabin). In this communication they will be called, following the terminology of Ferrata, basophil erythroblasts. The nucleus of the erythroblast has a tendency to form angular clumps of chromatin, giving a characteristic (sometimes called cartwheel) arrangement to the nuclear chromatin. This structure is of particular importance in identifying young erythroblasts.

The origin of the erythroblast is much disputed. Löwit and Denys<sup>10</sup> found that the earliest forms lack hemoglobin and resemble lymphocytes. This view, first denied by Bizzozero, is now generally accepted. The immediate precursor of the erythroblast appears to be mostly a large, round cell with strongly basophil, homogeneous cytoplasm, having a large vesicular nucleus, and resembling closely the younger forms of the erythroblast. The primitiveness of this cell is also indicated by the presence of nucleoli. The name proerythroblast was given to them by Ferrata and de Negreiros-Rinaldi;<sup>11</sup> "megalo blast" (Naegeli) is frequently used in a similar sense. Maximow recognized the existence of proerythroblasts in mammals, but erythroblasts in the bird are shown in his work<sup>8</sup> as derivatives of large lymphoid cells, called by him, with Ferrata, "hemocytoblasts."<sup>12</sup> The main difference between proerythroblasts and their precursors, the hemocytoblasts, is that the cytoplasm of the former is even more basophilic than the cytoplasm of the latter, and that it has a slightly less basophilic perinuclear area. Furthermore, the structure of the nucleus has already some of the characters of the erythroblast. Both possess nucleoli.

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10. Denys, J.: *Cellule* **4**:200, 1888.

11. Ferrata, A., and de Negreiros-Rinaldi: *Virchows Arch. f. path. Anat.* **215**:77, 1914.

12. The terms lymphoidocyte (Pappenheim, A.: *Folia haemat.* **5**:511, 1908) and erythrogonia (Helly, K.: *Beitr. z. path. Anat. u. z. allg. Path.* **49**:15, 1910) refer to similar cells.



Beyond the erythroblastic stage the erythropoiesis of the bird is not definitely known. Aside from the view of Maximow already mentioned, according to which erythroblasts are derived from the assumed multipotent large lymphocytes (hemocytoblasts), the idea supported by Sabin and her co-workers found wide acceptance, namely, that erythroblasts originate from endothelial cells. The origin of erythroblasts from endothelium during early embryonic life seems beyond dispute.<sup>7</sup>

#### MATERIAL OF STUDY

In the course of our study of the transmissible leukemia of fowls, erythroleukosis or a combination of erythroleukosis and myeloid leukosis developed in about half of the inoculated birds. Moreover, several spontaneous cases of erythroleukosis have been observed. One of these was found in association with sarcoma,<sup>13</sup> and another similar case occurred among the fowls inoculated with leukemic organs. In one bird repeated injections of benzene were followed by the development of erythroleukosis, and in another partial destruction of the bone marrow was followed by a similar event. Erythroleukosis was found in one chicken exposed to the ectoparasites of a leukemic fowl. These and a few atypical cases, most of them closely followed from the onset of illness until death, are the subject of this paper. In addition, a few experiments will be described that were performed to produce severe anemia by repeated bleeding or by continued administration of blood poisons, and the difference between these types of secondary anemia and erythroleukosis will be discussed.

#### TECHNIC

*Blood Smears.*—Blood smears were taken from all birds that appeared pale or ill, and at irregular intervals (from one to three months) from the entire flock.

*Blood Counts.*—Blood counts were made by diluting blood 1:100 or 1:200 with Toisson solution and counting white and red cells in the same chamber. The counting of normal blood and of pathologic blood other than erythroleukotic is satisfactory by this method. In erythroleukosis there is a continuous transition between red cells and their lymphoid precursors, making the counting of white cells arbitrary and somewhat inaccurate, the lymphoid erythroblasts being thus counted with the white cells. A more accurate method in these cases consists of determining the total number of cells and calculating the number of the various constituents on the basis of the differential counts. With some experience, however, direct counting, a much simpler procedure, gives approximately accurate results.

Recently several investigators described new methods of counting the blood cells of fowls. One of them<sup>14</sup> said that when suspended in Toisson solution the

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13. Dr. E. L. Stubbs gave me permission to describe this case.

14. Shaw, A. F. B.: J. Path. & Bact. **33**:833, 1930.

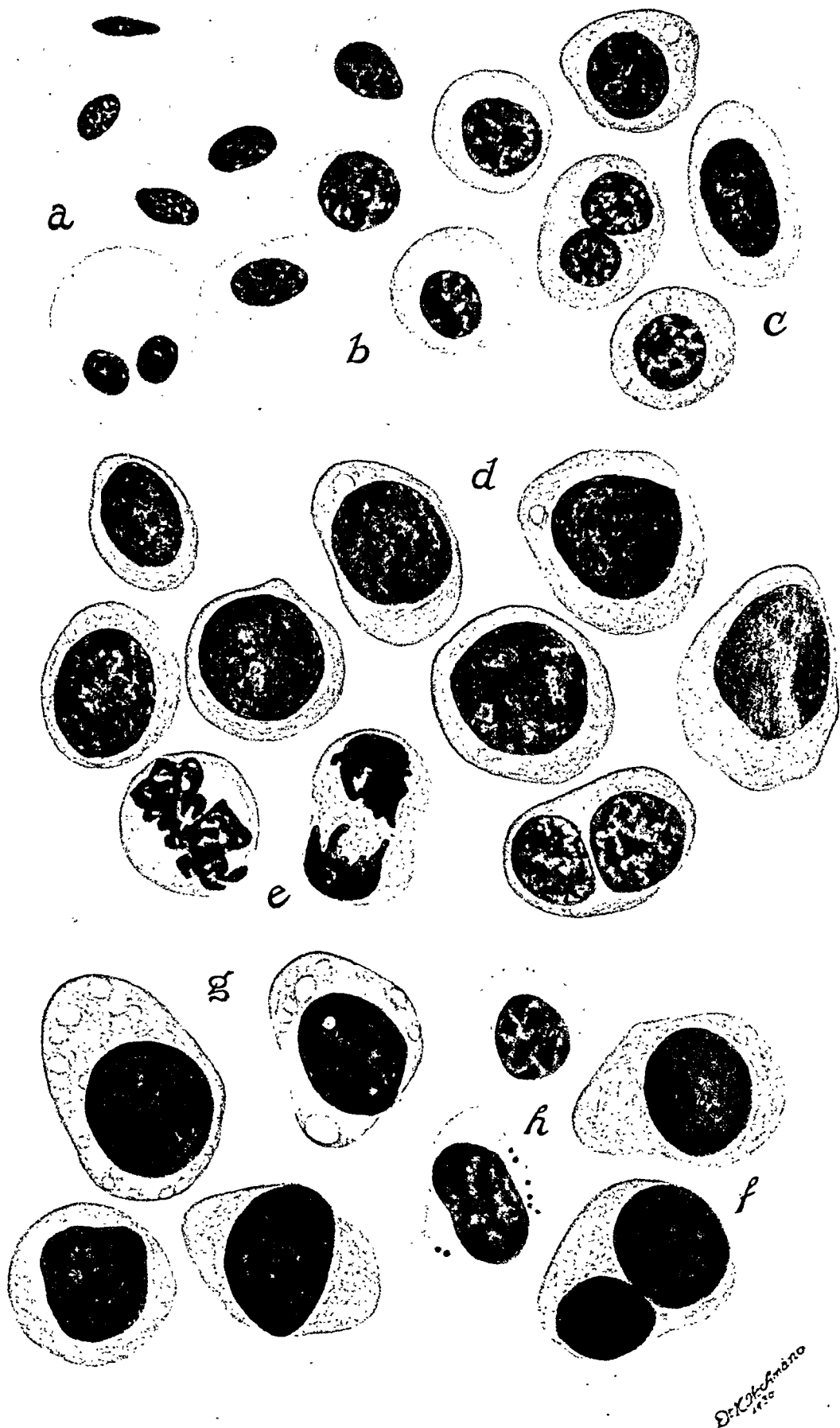


Fig. 1.—The blood cells in erythroleukosis of the fowl; *a*, erythrocytes; *b*, polychrome erythrocytes; *c*, polychrome erythroblasts; *d*, lymphoid erythroblasts; *e*, mitotic figures; *f*, lymphoid cells; *g*, lymphoid cells with vacuoles, and *h*, small lymphocytes.



lymphocytes of the fowl cannot be differentiated from the thrombocytes, but in my experience the differences between them have been marked. The thrombocytes are spindle-shaped not unlike erythrocytes, but they are somewhat smaller, lack hemoglobin and appear very pale in the counting chamber. The lymphocytes are much smaller and round, and take up crystal violet intensely. The thrombocytes were left entirely out of consideration in this study.

*Smears from Organs.*—Pieces of organs were cut in chicken serum and the larger particles allowed to settle. A smear was made with a small drop of the turbid suspension of cells, as is done with blood droplets. This method served the purpose of comparing the cells present in the blood-forming organs with those of the blood, and also permitted a rapid tentative diagnosis of some tumors.

*Other Procedures.*—For staining of slides, fixation of organs, staining of sections and nomenclature used, see an earlier publication by Dr. Furth.<sup>15</sup>

#### ERYTHROLEUKOSIS PRODUCED BY TRANSMISSION

Table 1 is a survey of the cases of erythroleukosis occurring among the fowls inoculated with leukemic material in the course of the experiments in transmission of this disease already described.<sup>15</sup> The number of the bird and that of the series are given to facilitate a correlation of the data presented.

*Symptomatology.*—The first signs of illness were discovered from 13 to 124 days after inoculation, with an average of 47 days. In most instances slight paleness attracted attention, while the bird still appeared active and the breast muscle was fairly strong. In a few instances, however, the comb was red, and only the routine examination of the blood led to the discovery of the pathologic condition. These cases are of particular interest because they indicate that erythroleukosis does not arise from preceding anemia. That the onset of spontaneous erythroleukosis may be similar is shown in case 366 (p. 22).

Many of the fowls died shortly after the disease became manifest while apparently in good physical condition, but others lost weight and became extremely emaciated. The duration of the illness averaged 19 days, and the birds lived from 21 to 204 days after inoculation. In several instances death was caused by an intercurrent disease, such as purulent infection of the upper respiratory tract or of the air sacs. Recovery was observed in two cases, in which the diagnosis was based solely on examinations of the blood.

*Blood Picture.*—The principles followed in differentiating immature erythrocytes have been described. Figures 1 and 2 illustrate the various types of cells of the erythroblastic series. A peculiar large lymphoid cell (figs. 1 *f* and *g* and 2 *E*) has been included, because it has not been seen in conditions other than erythroleukosis. The nucleus is large and vesicular and appears to be homogeneous. Nucleoli

15. Furth, J.: J. Exper. Med. 53:243, 1931.

TABLE 1.—Erythroleukosis and Anemia Among *Forols* Inoculated with *Leukemic Material*

Chickens	Series	Weight, Gm., at Time of Inoculation		Material Used for Transfer	Period of Incubation, Days	Duration of Illness, Days	Length of Life after Inoculation, Days	Summary of the Examination of the Blood *
		Inoculation	Death					
177	I	920	1,150	Organ emulsion 198	79	2	81	Erythroleukosis (erythroblastic), incomplete osteosclerosis
178	IV-J	1,310	850	Blood 365; myeloid leukosis	69	16	85	Erythroleukosis (atypical)
269	IV-J	1,450	1,450	Blood 365; myeloid leukosis	69	13	82	Erythroleukosis (atypical)
270	II-A	920	1,110	Blood 177; erythroleukosis	43	24	67	Erythroleukosis (erythroblastic)
276	II-A	850	1,280	Blood 177; erythroleukosis	132	72	204	Erythroleukosis (erythroblastic and lymphoid), with osteosclerosis
328	III-A	660	.....	Organ 266; myeloid leukosis	69	1	70	Erythroleukosis (erythroblastic)
335	III-B	940	.....	Organ 266; myeloid leukosis	...	..	169	Erythroleukosis (erythroblastic), with mild myeloid leukosis
455	IV-B	1,040	.....	Blood 328; erythroleukosis	114	19	133	Erythroleukosis (erythroblastic), mild
466	IV-D	1,100	1,775	Plasma 361; myeloid leukosis	71	16	87	Anemia secondary to myeloma
489	IV-C	980	1,230	Blood 361; myeloid leukosis	90	9	99	Erythroleukosis (mainly erythroblastic)
499	IV-O	710	1,170	Berkfeld filtrate 361; myeloid leukosis	100	91	191	Erythroleukosis (lymphoid, changing to erythroblastic)
511	IV-J	910	660	Blood 365; myeloid leukosis	46	14	60	Erythroleukosis (erythroblastic), with myeloid leukosis
553	V-B	730	.....	Organ emulsion 399; lymphoid leukosis	117	29	146	Erythroleukosis (lymphoid), recovered
565	V-C	935	850	Bone marrow 505; myeloid leukosis	131	21	154	Erythroleukosis (erythroblastic), with myeloid leukosis
575	IV-G	1,030	.....	Blood 365; myeloid leukosis	64	17	60	Erythroleukosis (lymphoid), with mild myeloid leukosis
599	III-C	870	.....	Blood 276; erythroleukosis	20	11	31	Erythroleukosis (lymphoid)
602	III-C	690	640	Blood 276; erythroleukosis	28	10	38	Myeloid leukosis, with erythroleukosis (lymphoid)
604	III-C	800	650	Blood 276; erythroleukosis	26	4	30	Erythroleukosis (lymphoid), with myeloid leukosis
605	III-C	800	820	Blood 276; erythroleukosis	26	3	29	Erythroleukosis (lymphoid)
612	IV-H	750	.....	Blood 276; erythroleukosis	44	4	48	Erythroleukosis (erythroblastic)
614	IV-H	700	.....	Blood 276; erythroleukosis	28	14	42	Myeloid leukosis, with erythroleukosis (erythroblastic)
615	IV-H	740	.....	Blood 365; myeloid leukosis	56	39	95	Erythroleukosis, mild
627	IV-H	840	.....	Frozen and thawed blood 365; myeloid leukosis	44	2	46	Erythroleukosis (lymphoid)
647	V-C	700	750	Blood 490; myeloid leukosis after treatment with distilled water	124	26	150	Erythroleukosis (erythroblastic), with myeloid leukosis
637	IV-J	740	660	Blood 365; myeloid leukosis	16	17	33	Erythroleukosis (erythroblastic)
633	IV-J	840	900	Blood 365; myeloid leukosis	32	1	33	Erythroleukosis (lymphoid)
655	IV-J	700	555	Blood 365; myeloid leukosis	43	23	67	Erythroleukosis (erythroblastic), with myeloid leukosis
656	IV-J	700	975	Plasma 365; myeloid leukosis	35	5	40	Erythroleukosis (lymphoid)
661	IV-J	680	940	Plasma 365; myeloid leukosis	33	6	41	Erythroleukosis (lymphoid)
670	IV-J	900	.....	Frozen and thawed blood 365; myeloid leukosis	25	4	29	Erythroleukosis, mild
675	IV-J	845	1,070	Blood 365 after treatment with distilled water	113	7	129	Erythroleukosis (erythroblastic), with sarcoma
681	IV-J	790	800	Blood 365 after treatment with distilled water	43	12	55	Erythroleukosis, with myeloid leukosis
691	IV-L	920	.....	Blood 365; myeloid leukosis	20	1	21	Erythroleukosis, incipient
694	IV-L	680	700	Blood 365; myeloid leukosis	16	9	25	Erythroleukosis (erythroblastic)
698	IV-M	720	1,000	Blood 604; myeloid leukosis	29	20	49	Erythroleukosis (erythroblastic)
705	IV-M	1,580	1,140	Plasma 604; myeloid leukosis	55	9	64	Erythroleukosis (erythroblastic), with myeloid leukosis
715	IV-N	770	.....	Blood 604; myeloid leukosis	27	23	55	Erythroleukosis, recovered
723	IV-N	800	640	Plasma 604; myeloid leukosis	24	31	55	Erythroleukosis (erythroblastic)
739	VI-A	900	500	Blood 572; myeloma	51	26	80	Erythroleukosis (erythroblastic), with myeloid leukosis
757	V-F	770	.....	Blood 499; erythroleukosis	23	11	134	Erythroleukosis (erythroblastic)
759	V-F	840	750	Blood 499; erythroleukosis	22	32	54	Erythroleukosis (erythroblastic)
767	V-F	590	550	Plasma 499; erythroleukosis	28	27	55	Erythroleukosis (erythroblastic)
795	IV-O	850	750	Blood 600; erythroleukosis, myeloid leukosis	13	9	22	Erythroleukosis (lymphoid)
798	VI-B	830	.....	Blood 643; myeloid leukosis	67	1	68	Erythroleukosis (lymphoid)

\* The type of erythroleukosis (lymphoid or erythroblastic) refers to the dominating cell in the blood stream.

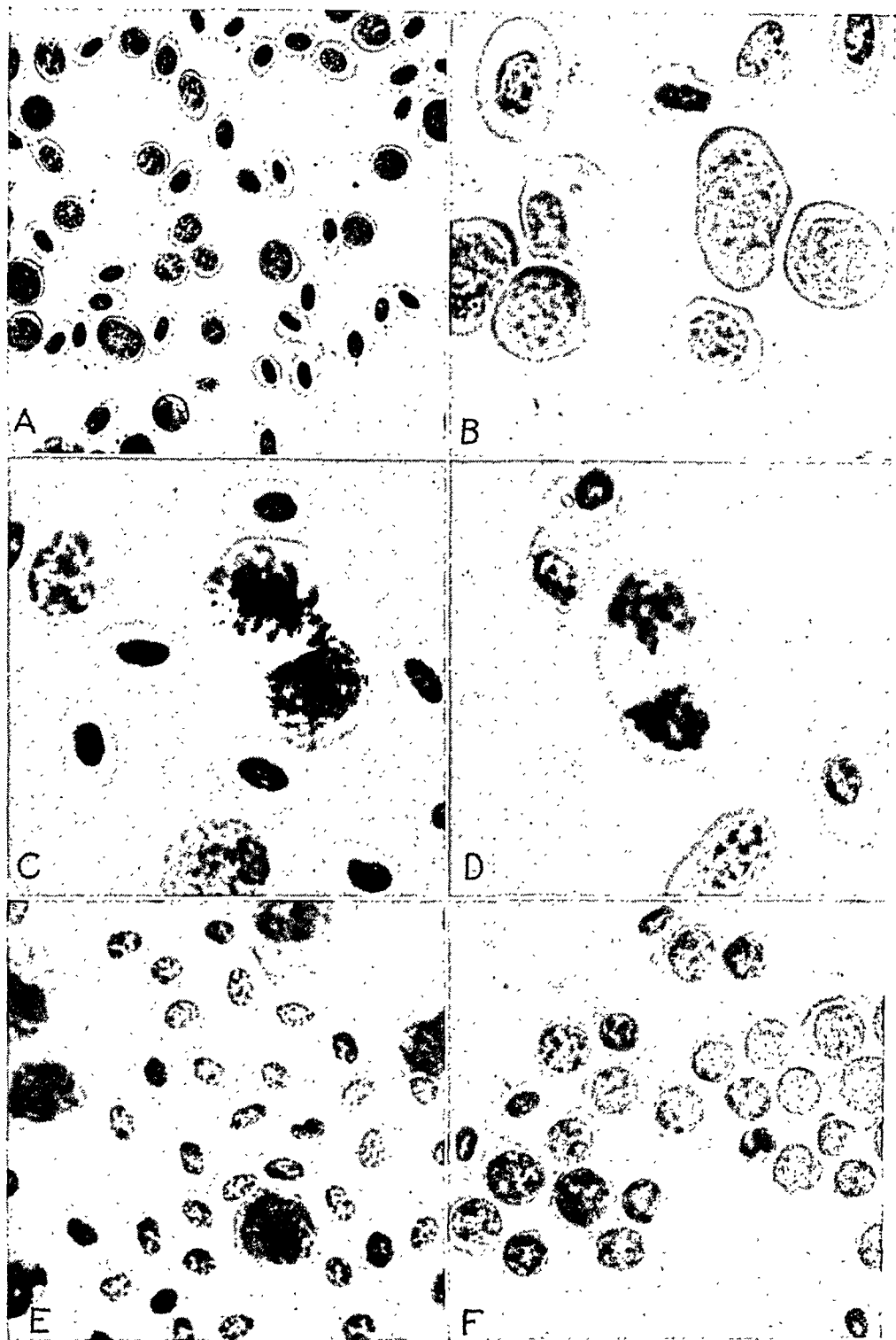


Fig. 2.—*A*, a blood smear in erythroleukosis of the erythroblastic type; *B*, basophil erythroblasts shown with higher magnification; about  $\times 1,000$ ; *C*, one mitotic figure, one basophil erythroblast, one lymphocyte and one polymorphonuclear leukocyte with rod-shaped granules; *D*, mitotic figure; *E*, blood smear in erythroleukosis of the lymphoid cell type, showing one lymphoid erythroblast and five lymphoid cells; *F*, smear from the liver in erythroleukosis, showing the erythroblastic character of the lymphoid cells of the capillaries of the liver.

TABLE 2.—Blood Picture in Erythroleukosis Produced by Transmission

Date of Examination	White Blood Cell Count in 1000's	Red blood Cell Count in 1000's	Hemoglobin (Sgdl)	Erythrocyte#	Polychrome Erythroblasts	Basophil Erythroblasts	Proerythroblasts	Primitive Lymphoid Cells	Same with Vacuoles	Mitotic Figures	Large Mononuclears	Promyelocytes	Myelocytes	Metamyelocytes	Polymorphonuclears	Mast Cells	Lymphocytes	Chicken	Differential Count											
																			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets	Atypical Neutrophils
177 3/26-6/14*	Normal 57	445	15	Few	Abundant	23	4	3	1	..	12	1	9	..	25	1	62	177	3/26-6/14*	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
178 1/16-1/11	Normal 15	1,360	32	Many	Few	9	2	5	1	..	14	..	..	..	50	5	36	178	1/16-1/11	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	12.5	830	19	Many	Few	5	5	12	13	..	25	..	..	..	11	4	46			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	Low normal	.....	..	Abundant	Few	..	2	16	12	..	11	..	..	..	8	..	47			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
269 1/16-4/8	High normal	.....	..	.....	.....	..	..	1	..	..	15	..	..	..	73	1	10	269	1/16-4/8	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	2/27/30	.....	12	Few	Few	3	..	..	..	..	11	..	..	..	25	6	60			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	Normal	690	12	Many	Few	5	4	4	3	..	6	1	..	..	19	6	47			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	45	410	8	Many	Many	14	..	13	2	..	15	4	1	..	45	..	8			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	38	287	9	Many	Few	2	..	12	1	1	16	3	..	..	54	3	8			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
276 6/11-1/4	Normal	.....	46	Abundant	.....	16	..	..	..	..	5	..	..	..	26	10	59	276	6/11-1/4	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	20	2,095	..	Few	Many	34	13	2	1	..	15	1	..	..	2	1	61			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	81	355	13	.....	Abundant	..	..	10	33	1	6	..	..	..	1	..	41			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
328 7/26-1/4	Normal	2,080	..	.....	.....	..	..	..	..	..	19	..	..	..	36	4	41	328	7/26-1/4	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	41	1,280	30	Few	Abundant	42	10	15	7	..	25	..	..	..	37	2	36			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	14	.....	..	.....	.....	..	..	..	..	..	4	..	..	1	15	..	6			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
358 8/29-2/14	Normal	.....	..	Many	Abundant	30	3	3	6	..	16	7	2	2	31	6	47	358	8/29-2/14	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	High normal	.....	..	.....	Abundant	..	..	..	..	..	21	..	..	..	5	..	21			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
469 10/18-4/18	Normal	2,290	..	Few	Few	5	1	16	41	..	8	..	..	..	43	1	48	469	10/18-4/18	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	48	1,015	30	Few	Many	5	4	35	30	..	3	..	..	..	2	2	30			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	57	1,128	..	Many	Abundant	25	12	19	13	2	1	1	..	..	1	..	24			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	214	570	14	Few	Few	11	11	34	23	..	2	..	..	1	15	..	25			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	13	2,835	49	Few	Few	2	..	..	..	..	6	..	..	..	14	5	73			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
553 12/10-7/7	Normal	.....	..	Very few	.....	..	..	..	..	..	12	..	..	..	33	5	50	553	12/10-7/7	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	36	1,250	29	Very few	Very few	1	1	22	1	..	9	2	..	..	1	3	60			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	68	1,160	..	Many	Many	10	1	..	..	..	3	1	..	..	16	6	63			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	Normal	.....	..	Many	Very few	..	3	8	..	..	8	..	2	..	14	4	74			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	Normal	.....	..	Many	Few	3	1	1	..	..	6	..	..	..	21	5	54			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	Normal	.....	..	Few	Few	5	..	1	..	..	16	1	1	..	28	9	73			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	High normal	.....	..	.....	.....	..	..	..	..	..	21	..	..	..	29	..	50			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
575 2/26-3/17	High normal	.....	..	Few	Few	5	..	1	..	..	14	..	..	..	23	6	52	575	2/26-3/17	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	17	2,145	41	Very few	Few	6	2	1	..	..	12	..	..	..	9	3	66			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	3/3	1,005	20	Few	Few	5	..	21	2	..	14	3	..	..	..	5	48			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	26	770	..	Many	Many	11	8	20	3	2	17	..	6	..	..	18	18			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	95	.....	..	.....	.....	..	..	..	..	..	5	..	..	..	19	5	71			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	Normal	.....	..	.....	.....	16	1	5	..	..	7	..	..	..	19	5	71			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
602 1/4-2/11	Normal	.....	..	Few	Many	16	1	..	..	..	5	..	..	..	5	1	64	602	1/4-2/11	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets
	55	1,380	23	Very few	Very few	..	..	9	25	..	49	1	2	..	4	..	7			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets	Atypical Lymphocytes	Atypical Monocytes	Atypical Eosinophils	Atypical Basophils	Atypical Platelets





are present. The cytoplasm is narrow and very basophil, appears round and often has small lingulate processes. The extreme basophilia differentiates these cells from hemocytoblasts, and lack of the characteristic nuclear structure, from proerythroblasts. In a large percentage vacuoles are seen. In fixed and stained sections differentiation is difficult, if not impossible.<sup>16</sup> These cells are here given temporarily the designation "lymphoid" cells, and they are tabulated as immediate precursors of proerythroblasts, although this genetic reconstruction based on morphologic grounds requires further evidence. It would seem possible that many of these cells are lymphoid erythroblasts that have undergone degeneration, hence the structureless nucleus and vacuolization of the cytoplasm. Direct smear from the infiltrated organs would seem to support this view (p. 14).

The blood cells of healthy fowls will not be reviewed here. The large mononuclear cells seen in myeloid leukemia will be called, with Ellermann, "large mononuclear cells (myeloblasts)" and similar cells with purple granules "promyelocytes." They will be described more fully in a paper on myeloid leukosis. Some uncertainty as to the correctness of this nomenclature should be kept in mind.

At the onset of the disease, in contrast with secondary anemia, there appears to be an adequate number of erythrocytes in the circulation, and the amount of hemoglobin is likewise not considerably diminished; e. g., in fowl 757 there were 2,850,000 red blood cells at the onset of disease; the red blood cells in fowl 750 numbered 2,000,000 even before death. Polychromatophil erythrocytes are present in moderate amounts, whereas erythroblasts, among them the early forms, are relatively numerous. In some instances lymphoid cells appear in the circulation in considerable numbers, while anemia is extremely slight (e. g., fowl 681).

The blood picture during the course of illness is given in table 2. Since there is a continuous series between erythrocytes and their lymphoid precursors, the differential counts given naturally are arbitrary and somewhat inaccurate.

The medium or large lymphoid cells are counted with the white cells, and if they appear in considerable numbers the blood picture becomes leukemic. This study includes eleven such cases with fatal outcome, which in table 1 are designated as "erythroleukosis (lymphoid)" to distinguish this from the more common erythroblastic form. In seven of these cases the figures for white cells were: 42,000 (798), 67,000 (605), 95,000 (575), 101,000 (661), 164,000 (579), 182,000

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16. Maximow (footnote 8, fig. 79).

(656) and 201,000 (602). The dominating cell was the lymphoid cell described. Thus erythroleukosis of the lymphoid cell type may be distinctly leukemic.

When these cases are considered separately, it becomes evident that the period of incubation (thirty-six days) is below the average, and that their course is more fulminating, the average length of illness being seven days. This type may therefore be understood as a more acute form of erythroleukosis.

It is obvious from table 2 that a sharp line does not exist between erythroleukosis of the lymphoid cell type and erythroleukosis of the erythroblastic type. Indeed the continuous transitional forms between the two support the assumption that they are varieties of the same process. A change of type from erythroblastic to lymphoid (e. g., 575 and 602) or the reverse (499) may likewise occur. In microscopic anatomy of organs no marked difference is observed between these pathologic conditions.

Ellermann's statements that the leukocytes are outside the essential change of erythroleukosis, and that, if the "lymphoidocytes" are not considered, one gets a normal percentage of each of the various types of white cells, are not supported by the data presented in table 2. A considerable decrease of the polymorphonuclear leukocytes appears to be the rule, and agranulocytosis is not uncommon. The behavior of the lymphocytes is less constant, but their number likewise decreases considerably in almost every pronounced case.

The presence of myelocytes is not uncommon in erythroleukosis. It is seen from table 2 that myelocytes were not found in smears taken before inoculations, but when blood smears were taken repeatedly after inoculation an occasional myelocyte was seen in almost two thirds of the cases of erythroleukosis, usually at a more advanced stage. Occasionally the number of myelocytes and large mononuclear cells (myeloblasts, Ellermann) became considerable. In most of these cases the sections of the blood-forming organs likewise revealed an involvement of both erythropoietic and granulopoietic systems. Indeed in several instances the microscopic picture of the bone marrow revealed a myeloid involvement not suspected after the examination of blood smears. In a few instances the blood picture at an early stage was that of erythroleukosis, which then changed completely to myeloid leukemia (e. g., 696).

Erythroblasts with double nuclei were described by Ellermann and were considered by him as pathognomonic for erythrocytes and erythroblasts. In figure 1 such cells are reproduced. It is noteworthy that cells with double nuclei were particularly numerous among the primitive lymphoid cells described, pointing to a connection with cells of erythro-

blastic potencies. Occasionally, however, double nuclei were found in cells other than those of the erythroblastic series.

Maximow<sup>17</sup> considered the erythroblast with a double nuclei a degenerated cell. In the microscopic aspect of fixed and stained smears there is no support for this opinion, for this cell appears to differ from the normal erythroblast merely by the double nuclei. It is more probably the result of an atypical division of a cell.<sup>18</sup>

Characteristic inclusions are found not infrequently in large mononuclear cells of myeloid leukemia, and will be described in a subsequent paper. They are rare in erythroleukosis.

Mitotic figures are found in practically every severe case of the transmissible leukosis. In some they are abundant. Figures 1 *c* and 2 *C* and *D* show some of the types seen in erythroleukosis. The large number of mitotic forms in the peripheral blood and in the capillaries of the organs offers the most direct support for the possibility, thus far surprisingly disregarded, that transfer of leukemia of fowls, not unlike transmission of cancer and leukemia of mammals, is bound to tumor cells.

*Anatomic Changes.*—Bone Marrow: As in myeloid leukemia the bone marrow was involved in all cases of erythroleukosis observed (cf. Schmeisser<sup>19</sup>). However, whereas myeloid leukemia seems to be preceded by an aleukemic stage with maximum myelocytic and myeloblastic hyperplasia of the marrow, the involvement of the peripheral blood in erythroleukosis appears to take place at a very early stage. The disease was diagnosed or suspected before death by reason of the erythroblasts present in the circulation in all cases, with the possible exception of one (503), which will be fully described in later paragraphs. The early involvement of the peripheral blood is probably due to the intravascular nature of erythrogenesis, whereas myeloid leukemia begins in the trabeculae of the marrow. The pathologic changes in erythroleukosis were most marked in the marrow, and in a few cases (178, 269 and 358) the specific involvement of the other organs was very mild, suggesting that the bone marrow is the primary site of erythroleukosis as it is of myeloid leukemia.

The gross appearance of the marrow is characteristic, though it was neither recognized with certainty in all cases nor always differentiated from the marrow of myeloid leukemia. The marrow shows loss or absence of fat. It is reddish or pale grayish, and its consistency is increased, so that it can be removed as a semisolid cylindric mass.

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17. Maximow (footnote 8, p. 388).

18. McGowan (footnote 3, p. 71).

19. Schmeisser, H. C.: Johns Hopkins Hosp. Rep. **17**:551, 1916.

In sections the latent and patent blood vessels can be well made out in the early or in the mild cases. The former vessels show sinusoid dilations and are filled with erythroblasts and lymphoid cells, the latter contain chiefly mature erythrocytes and immature cells such as are seen in the peripheral circulation. The structure of the marrow is preserved (fig. 3 *C*). In the trabeculae, foci of small cells resembling

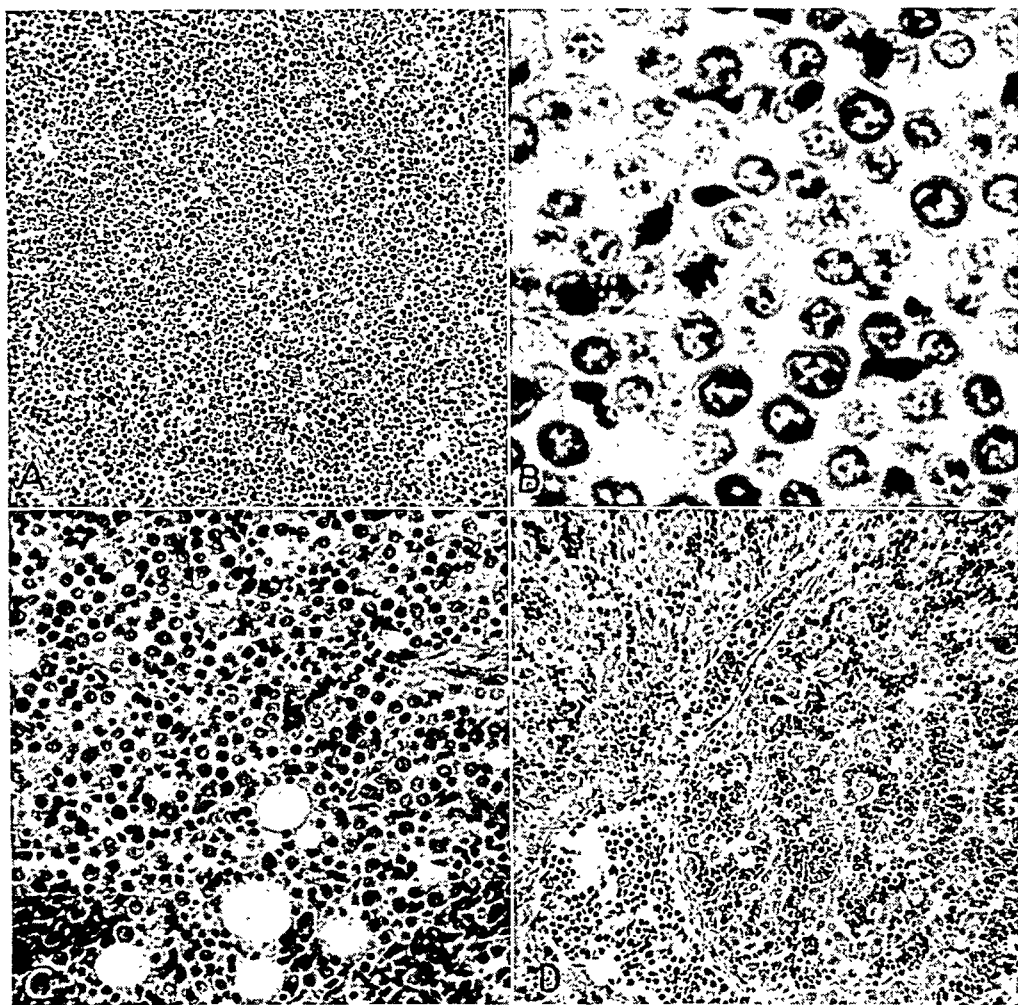


Fig. 3.—*A* and *B*, the bone marrow in advanced erythroleukosis of the fowl; *C*, the bone marrow in incipient erythroleukosis; *D*, the liver in erythroleukosis.

lymphocytes and foci of myelocytes are seen, the latter sometimes somewhat in excess of normal. Figures that could be interpreted as proliferating endothelial cells have not been identified with certainty. As the disease progresses, the crowding of the sinusoids becomes more pronounced, the fat content of the marrow becomes reduced, the trabeculae become narrower, and in an advanced stage the marrow has the appearance of a dense mass of lymphoid cells (fig. 3 *A* and *B*).

These observations are based on examination of the marrow of the femur and tibia. In the air-containing humerus, which was not systematically examined, no change was observed.

Liver: The liver is usually enlarged, but not to the same extent as in lymphoid leukosis. The average weight of the liver of the normal fowl is 2.8 per cent of the weight of the body (Pickens<sup>20</sup>). In twenty-nine cases of erythroleukosis the weight of the birds post mortem varied between 550 and 1,450 Gm.; the average weight was 863 Gm. The weight of the liver varied between 29 and 184 Gm., with an average of 64.5 Gm. (7.5 per cent of the weight of the body). In individual fowls, however, including all mild and some pronounced cases, the liver appeared to be of normal size (178, 269, 358, 503, 511, 575, 602, 605, 612, 613 and 691).

The color of the liver is in most instances distinctly grayish; in a very few instances it is slightly yellowish and fatty. Its consistency is as a rule slightly increased, but occasionally it is very brittle. The surface and cut surface either is homogeneous or shows a fine mottling caused by minute grayish areas.

Thus on gross examination of the liver erythroleukosis can in some instances not even be suspected. The microscopic appearances, however, are characteristic and pronounced in all but a few atypical or mild cases (178, 269, 358, 455 and 691). This specific involvement consists of a selective accumulation of lymphoid cells in the capillaries to a varying degree (fig. 3 *D*). Their location is intravascular, with the exception of an occasional tumor-like accumulation of lymphoid cells. In view of the fact that hemorrhages are found regularly in erythroleukosis, one might assume that such growth is perhaps due to lesions in the endothelial linings of the blood vessels. Associated small foci of myelocytes and myeloblasts, chiefly perivascular, are not uncommon. The character of the myeloid tissue in the livers of fowls will be discussed more fully in a subsequent paper on myeloid leukosis.

To ascertain the character of the lymphoid cells accumulating in the capillaries of the liver, smears were prepared from emulsions of the liver in chicken serum. The cells in such smears resemble lymphocytes very closely, varying greatly in size from that of the small to that of the large lymphocyte. The cytoplasm surrounds the nucleus in the form of a rather regular, narrow, basophil ring, with a clear halo around the nuclear membrane. The nucleus has a distinct network of chromatin, caused by a nodular or linear concentration of the chromatin; the characteristic sharply angular network of the erythroblasts is wanting. Nucleoli are distinct in many of these cells. They are surrounded by a denser mass of chromatin and are, if pronounced, slightly bluish (fig. 2 *F*).

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20. Pickens, E. M.: Rep. New York State Vet. Coll., 1917, p. 226.

Although such cells, notably those of smaller size, resemble lymphocytes very closely, the majority of the well preserved cells can be distinctly recognized as lymphoid erythroblasts. The term erythrogony (Helly<sup>21</sup>) would seem to be well chosen for such cells, which appear to be different from large lymphocytes (lymphoblasts).

Spleen: Enlargement of the spleen occurring in most instances to an extent not observed in any other condition known, with moderate

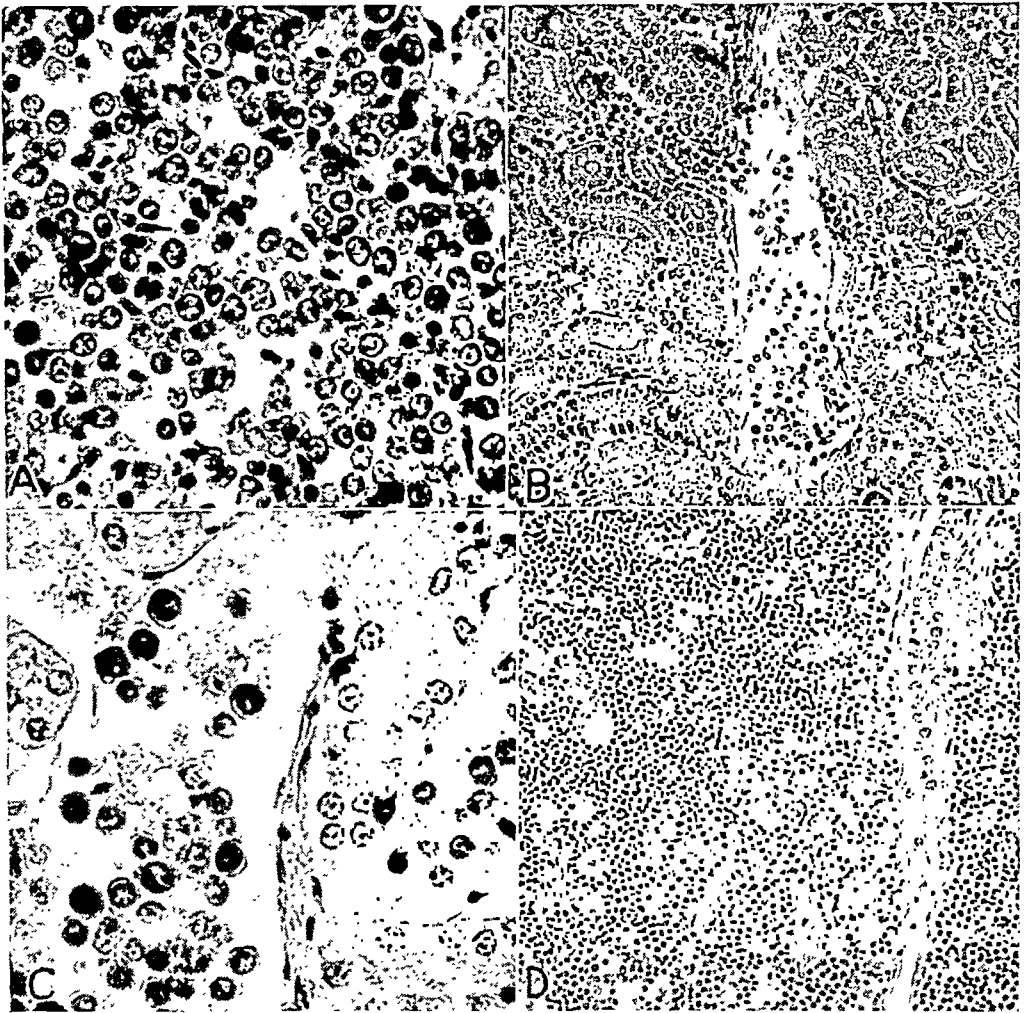


Fig. 4.—*A*, the spleen in erythroleukosis; *B* and *C*, the kidney in erythroleukosis; *D*, the difference in size and in character of the lymphoid cells seen in erythroleukosis and the lymphocytes of the thymus.

enlargement of the liver, appears to be the most striking feature of the gross postmortem examination. The size of the normal spleen is about 0.25 per cent of the weight of the body (Pickens<sup>20</sup>). In twenty-nine cases of erythroleukosis with an average weight of body of 863

21. Helly (footnote 12, second reference).

Gm., the weight of the spleen varied between 0.5 and 21 Gm., with an average of 6.1 Gm. (0.71 per cent of the weight of the body). This relatively low average is due to the fact that in some mild cases the spleen was only slightly enlarged and to the fact that in the atypical cases (178 and 269) it was below normal (0.05 per cent and 0.06 per cent of the weight of the body).

The color of the spleen is grayish brown, uniformly or with a fine mottling of minute pale grayish areas. It is moderately firm.

The involvement of this organ seems to begin by the accumulation of lymphoid cells in the pulp until by progressive accumulation they encroach on the follicles (fig. 4). This process is comparable to the compression of the granulocyte-containing trabeculae of the marrow by the proliferating lymphoid cells in the sinusoids (Ellermann<sup>1</sup>).

Spontaneous rupture of the enormous spleen was the immediate cause of the death of fowl 698.

Thus stasis of the lymphoid cells in capillaries is the dominant feature of erythroleukosis. This, although most constant in the blood-forming organs, is by no means limited to them.

Other Organs: Of the other organs, the kidney (fig. 4 *B* and *C*) and adrenal gland are frequently affected to a moderate degree. The involvement of the lung and heart is likewise common, but less extensive. In mild cases the stasis is localized to parts of the organs, depending apparently on the sluggishness of the flow of blood in these areas. Less frequent and less extensive is the involvement of the thymus (fig. 4 *D*) and thyroid glands, and distinct stasis in the pancreas has been observed in only one severe case (661).

Among the organs rarely affected, the nervous system is of particular importance because of its frequent involvement in paralysis and lymphoid leukosis of the fowl (cf. Pappenheimer<sup>22</sup>). In only one case (that of fowl 651) did I see slight leukostasis in the lumbar nerve. The ovary, testes and ganglions were found to be free from leukostasis.

Hemorrhages, particularly in the muscles, are frequently seen post mortem. Secondary parenchymatous degenerative changes, particularly marked in the kidney, are common, but have not as yet been studied closely.

*Comment.*—A progressive growth of lymphoid erythroblasts appears to be the essence of erythroleukosis. It would seem that the bone marrow is the first organ where the stasis caused by the multiplying lymphoid cells becomes manifest. This is then followed by a similar change in the spleen and in the liver and involvement of the other

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22. Pappenheimer, A. M.; Dunn, L. C., and Cone, V.: *J. Exper. Med.* **49**:63 and 87, 1929.

organs. This sequence of events is probably connected with the rate and character of the flow of blood in various organs, leading to retention and multiplication of cells.

#### CASE HISTORIES

The histories of a few cases will be reported in illustration of erythroleukosis. The first two of these cases are typical as described and require no further comment.

*Erythroleukosis of Erythroblastic Type.*—Fowl 328 weighed 660 Gm. on July 20; it was inoculated intravenously with an emulsion of organ of fowl 268 M. On August 9, the white blood cells numbered 40,800 and the red blood cells 2,060,000; the differential count was normal (see table 2) on September 27, and 17. On October 3 the comb was slightly pale, but the blood smear appeared normal; on October 4 both red and white cells were decreased in number, and the blood smear showed pronounced erythroleukosis (see table 2). The bird died following the removal of 9 cc. of blood from a vein of the wing.

Postmortem examination showed a moderately emaciated fowl, with large amounts of clear, yellowish fluid in the peritoneal cavity. The thymus was not enlarged. Much clear, frothy liquid escaped from the lungs on slight pressure. The liver weighed 70 Gm.; it was brittle and reddish brown with a slight grayish hue. The spleen weighed 12 Gm. and was reddish brown. The kidneys were pale grayish brown. The mucosa of the duodenum was spotted with minute red areas. In the cecum numerous whip-worms were found. The bone marrow of the femur and tibia was grayish, and the consistency was increased. The humerus contained air.

Microscopic examination revealed that the capillaries of the liver were enormously distended and filled with lymphoid cells. In the portal tissue there was a mild infiltration with small lymphocytes. Myeloid metaplasia was absent. The hepatic cells were filled with small fat droplets; they appeared to be compressed and showed in some areas necrotic changes, caused apparently by leukostasis. The marrow was crowded with lymphoid cells located in apparently endothelial-lined spaces. Granulocytes and small lymphocytes were seen in very small foci only. The pulp of the spleen was likewise packed with lymphoid cells. Stasis was pronounced in the kidney and adrenal gland, but was remarkably absent from the ganglion attached to the adrenal gland.

*Erythroleukosis of Lymphoid Cell Type.*—On January 16 fowl 656 weighed 770 Gm.; it was inoculated intravenously with 2 cc. of plasma of fowl 365. On February 20, the number of white cells did not seem to be increased, but there were among them many large lymphoid cells (see table 2). Several erythroblasts, many of them of the early type, were seen on February 25; an enormous increase of large lymphoid cells (white blood count, 182,000), some with double nuclei, many with vacuolated cytoplasm, was observed, and occasional myelocytes. The bleeding time was greatly prolonged, the clotting of the blood being incomplete after one and one-quarter hours. (The blood of two control fowls clotted completely within three and one-half and four minutes.)

At autopsy the weight was 975 Gm. The breast muscle was strong, the comb and mucous membranes were very pale; there were numerous hemorrhagic areas in the muscles; a moderate amount of serous liquid exuded from the lung. The liver weighed 59 Gm., was grayish brown and moderately firm and showed numerous minute grayish streaks and spots. The spleen weighed 4 Gm., and was rela-



tively more enlarged than the liver; it was slightly firm, with a meaty consistency, pale and grayish; the follicles were just visible. The testes were small. The kidneys were pale and appeared slightly swollen. The bone marrow (of the femur and tibia) was pale, uniformly grayish brown, resembling the spleen in color. The consistency appeared to be slightly increased. Several minute hemorrhagic spots were found in the mucosa of the duodenum and to a less extent in the small intestine proper. There were several whip-worms in the cecum. The cecal follicles were much swollen, and their surface showed hemorrhagic spots.

Microscopically, the bone marrow was almost entirely composed of densely packed lymphoid cells. The capillaries of the liver were studded with similar cells, and in this organ there were a few small perivascular lymphocytic accumulations. In the pulp of the spleen, lymphoid cells were abundant. There were pronounced leukostasis in the kidney and marked stasis in certain areas of the thymus, duodenum and cecal follicle. In the latter two organs coccidia were numerous.

*Erythroleukosis with Myeloid Leukosis.*—Fowl 655 weighed 700 Gm. and was inoculated intravenously with 0.5 cc. of blood of fowl 365 M. on January 16. On February 28 a smear showed erythroleukosis (see table 2). On March 3 the breast muscle was strong, the bird active, the comb moderately pale with a slight yellowish hue; the white blood count was 33,000; the red blood count, 1,730,000; the hemoglobin, 25. The blood clotted completely in three minutes. In a smear were seen numerous erythroblasts, many of them primitive, several large lymphoid cells and occasional myelocytes. On February 6 there was no marked change. On March 17 a smear (see table 2) showed moderate anemia, the very immature forms wanting. The chicken died on March 24. It was emaciated, weighing 555 Gm. All the organs were pale; the thymus was very small. In the lung there were several areas containing mucopurulent material near the large bronchi, and also reddish-brown consolidated areas involving not over one tenth of the lung substance. The liver weighed 28 Gm.; it was reddish brown with a fine network of yellowish-gray areas. Its consistency appeared increased. The spleen weighed 1 Gm.; it was pale and grayish and the follicles were not visible. The bone marrow was uniformly pale grayish brown. The grayish color and increased consistency of this organ were the only appearances at gross examination suggesting leukemia.

On microscopic examination the marrow showed maximum hyperplasia formed by myelocytes, as well as by lymphoid cells. In the sinusoids there were also erythroblasts in all stages of maturation, with a dominance of the lymphoid forms. In the liver there were several small perivascular myelocytic foci, and there was slight stasis of lymphoid cells in the capillaries; some lymphoid cells formed also small extravascular foci. In the kidney and heart there was mild leukostasis. In the lung there were large areas of necrosis surrounded with inflammatory exudate.

Comment: This case is remarkable (1) for the disappearance of erythroblasts from the circulation about seven days before death, (2) for the inconspicuousness of the gross pathologic appearances in the liver and spleen, (3) for the intensity of the myeloid hyperplasia, which was combined with erythroleukosis, and (4) for the purulent infection of the lung, which might have been responsible for the atypical course.

*Erythroleukosis with Slight Myeloid Involvement.*—On January 16 fowl 681 weighed 790 Gm.; it was inoculated intravenously with 1 cc. of blood of fowl 465

after the blood had been treated with distilled water.<sup>15</sup> On February 28 a smear was suggestive of erythroleukosis (see table 2). On March 3 the comb was red with a faint yellowish hue, the breast muscle fairly strong, and the bird active. The white blood cells numbered 137,000 (see table 2); the red cells, 2,650,000. The hemoglobin was 42. In a smear erythroblasts were few; the picture was dominated by large lymphoid cells, several of which showed double nuclei and most of them vacuolization of the cytoplasm. On March 5 occasional mitotic figures and myelocytes were seen; the smear otherwise appeared as on March 3. On March 11 the white blood count was 315,000, the red blood count 1,040,000, the hemoglobin, 23; clotting of the blood was complete in ten minutes, and numerous large mononuclear cells were seen, similar to those observed in myeloid leukemia, some of them with large purple granules. Myelocytes and polymorphonuclear leukocytes were absent. Erythroblasts in all stages of development and primitive lymphoid cells were abundant. Mitotic figures were numerous. The fowl died on March 12.

At autopsy it was moderately emaciated, weighing 800 Gm. The comb was pale and slightly yellowish. The thymus was small and grayish. The lungs were pinkish and apparently normal. The heart was pale brown, with injected blood vessels. The esophagus and trachea were free. The liver weighed 71 Gm. and was distinctly grayish brown on the cut surface, with a fine grayish-white mottling. The spleen weighed 6.5 Gm.; it was slightly firm; the cut surface was grayish brown; the follicles were very minute. The bone marrow was uniformly grayish and resembled the spleen in color. The kidney was pale. The intestinal tract showed no marked abnormalities with the exception of the cecum, which showed a few minute hemorrhagic spots. The microscopic picture of the organs was that of pronounced erythroleukosis similar to that in case 328 described in foregoing paragraphs; myeloid involvement was slight and was localized in the liver and adrenal gland.

*Erythroleukosis.*—The development of leukemia in fowl 728 is well reproduced in table 2. The terminal blood picture suggested a myeloid complication, yet the microscopic picture of the organs was that of pure erythroleukosis.

*Atypical (Aplastic?) Erythroleukosis.*—Fowl 269 was first inoculated on Jan. 4, 1929, with material from fowl 177 E, with negative result. On Jan. 16, 1930, the bird (weight, 1,750 Gm.) was reinoculated with blood from fowl 365 M. The subsequent development of a blood picture suggesting erythroleukosis is given in table 2. The severe anemia, not observed to such an extent in typical cases of erythroleukosis, is noteworthy. Weakness of the leg was noted on April 3.

At autopsy, on April 8, the body weighed 1,450 Gm. and was moderately emaciated; the comb and organs had a pronounced icteric color. The liver was yellowish brown, and weighed 34 Gm.; the consistency appeared slightly increased. The spleen was small (0.75 Gm.). The bone marrow appeared to be partly yellowish gray and fatty, and partly grayish brown. The kidney was yellowish brown. The heart was somewhat dilated and flabby.

On microscopic examination the bone marrow showed advanced hyperplasia composed chiefly of densely packed young polymorphonuclear leukocytes and their precursors and to a less extent large lymphoid cells. In the capillaries of the liver, cells laden with yellowish-brown pigment were numerous. Leukostasis was indistinct and occurred to a very slight degree in a few capillaries only. In the periportal area there was a proliferation of large mononuclear cells with strongly

basophil cytoplasm and of myelocytes. In the spleen, which showed no specific involvement, pigment cells were abundant.

Comment: This case is noteworthy, for at gross examination there was no indication of the erythroleukosis suggested by the blood picture before death, and in the microscopic picture of the blood-forming organs the changes characteristic of erythroleukosis were indistinct. Since this and the similar case, in fowl 178, have not been transmitted it remains uncertain whether they were caused by the transmissible agent of the leukemia of fowls; their designation as erythroleukosis may be disputed.

*Erythroleukosis of Erythroblastic Type Ending in Recovery.*—The diagnosis in the case of fowl 715 was based on the examination of blood (see table 2).

*Erythroleukosis with Sarcoma.*—Fowl 675, on January 16, weighed 845 Gm.; it was inoculated intravenously with 0.5 cc. of blood of fowl 365 after the blood had been treated with distilled water. Blood smears taken repeatedly from February 20 until April 26 were negative. On April 29 a smear showed the picture of erythroleukosis. The bird died on May 6. On six successive days preceding death the fowl was fed with liver extract by Dr. Amano, who observed during this period a drop in the count of red blood cells to 1,000,000 and a simultaneous disappearance of the early erythroblasts. Hemorrhage from the liver was the immediate cause of death. The weight of the fowl post mortem was 1,070 Gm. The lung was pinkish, the heart pale and the kidney yellowish brown, but otherwise there were no marked changes. The liver was brittle and weighed 119 Gm.; it was mottled with numerous minute grayish-white nodules and contained several prominent whitish nodules varying in size from a few millimeters to about 3 cm., many of which were yellowish in the center. These nodes, unlike lymphoid tumors, did not emulsify easily and, when cut, formed small coherent particles. In the spleen, which was pale grayish and weighed 3.5 Gm., there was one tumor node of about 1 cm. across. A similar, but slightly firmer, tumor was found in the skin of the neck. The bones were somewhat soft, and in those containing marrow there was proliferation of fibrous bony tissue from the endosteum, in some places obliterating the cavity almost entirely. The marrow obtained from the femur was red; that from the tibia was greenish gray, the greenish color suggesting necrosis. The microscopic picture of the tumors of the spleen, liver and skin showed a great resemblance. They were formed by large, round, polymorphous, or less frequently somewhat elongated, cells and were diffusely infiltrated with leukocytes. In the tumor of the skin there was abundant reticulum. It contained numerous mitotic figures and infiltrated the muscle tissue diffusely. In the parts of the liver that were not grossly invaded by the tumor, lymphoid cells crowded the sinusoids and small foci of tumor cells and foci of myelocytes were seen. Leukostasis was not found in the kidney, adrenal gland and heart. and was indistinct in the spleen.

Comment: This and another similar spontaneous case (that of fowl 515), to be described in later paragraphs, give rise to the thought that erythroleukosis may be secondary to a tumor.

TABLE 3.—*Blood Counts in Spontaneous Erythroleukosis and Experimental Anemia*

Chicken	Date of Examination	White Blood Cell Count in 1000's	Red blood Cell Count in 1000's	Hemoglobin (Sahli)	Polychrome Erythrocytes	Polychrome Erythroblasts	Spontaneous Erythroleukosis	Polychrome Erythroblasts	Basophil Erythroblasts	Proerythroblasts	Primitive Lymphoid Cells	Same with Vacuoles	Mitotic Figures	Large Mononuclears	Promyelocytes	Myelocytes	Metamyelocytes	Polymorphonuclears	Mast Cells	Lymphocytes
235	4/10	24.7	540	15	Abundant	Abundant	Abundant	9	2	..	..	..	..	4	..	..	..	20	1	64
	5/10	Normal	.....	..	Abundant	Abundant	Abundant	8	1	..	..	1	..	13	1	1	..	4	2	74
	6/25	Normal	.....	..	Abundant	Abundant	Abundant	9	1	2	2	..	..	11	..	..	..	5	1	71
	9/7	Normal	.....	..	.....	.....	.....	..	..	..	..	..	..	13	..	..	1	54	..	32
	2/11	Normal	.....	..	Practically absent	Very few	Very few	11	2	8	8	10	1	17	..	..	..	4	1	46
441	2/26	39	2,695	53	Many	Numerous	Numerous	24	3	7	7	1	..	12	1	..	..	1	8	43
	5/2	Much increased	.....	..	Many	Abundant	Abundant	53	9	12	12	1	5	3	..	2	3	10	..	2
	5/21	930	560	13	None	None	None	..	..	95?	95?	..	1	1	..	1	..	2	..	..
	5/26	975	465	..	None	None	None	..	..	96?	96?	..	..	..	1	1	..	2	..	..
515	11/1	11	1,570	52.5	Abundant	Many	Many	16	..	17	17	1	..	13	11?	..	..	29	5	8
Experimental Anemia																				
245	4/16	22.5	1,040	20	Abundant	Abundant	Abundant	1	..	2?	2?	..	..	10	..	..	..	33	4	50
292	12/12	40.5	740	Less than 10	Abundant	Few	Few	..	..	..	..	..	..	23	..	..	..	68	4	5
	1/11	41	745	14	Abundant	Few	Few	..	..	..	..	..	..	26	..	..	..	70	..	4

The relation of the erythroleukosis to the osteosclerosis in which the marrow is partly or wholly obliterated by bone-needs further investigation.

#### SPONTANEOUS ERYTHROLEUKOSIS

There are no data available on the occurrence and incidence of spontaneous erythroleukosis among fowls. Those who have observed erythroleukosis did not distinguish it from myeloid leukosis or presumably considered it lymphoid leukemia.

Hare in cooperation with us found among 490 autopsies of fowls 22 spontaneous cases of leukosis, of which 3 resembled erythroleukosis. The data available on these cases are not sufficient for a full description. An "epidemic" of spontaneous erythroleukosis on a Pennsylvania farm is now being investigated by Biltz, Stubbs and myself.

Several cases of presumably spontaneous erythroleukosis have been observed in this laboratory among fowls not inoculated with leukemic materials. Their history will be fully described here.

*Case of Spontaneous Erythroleukosis.*—On Sept. 7, 1929, fowl 366 showed slight paleness, and on November 11, a relative lymphocytosis (for differential counts see table 3). On Feb. 11, 1930, a blood smear suggested incipient erythroleukosis. On February 26 the breast muscle was fairly well developed, the comb red, the weight 1,460 Gm.; the white blood cell count was 39,000, the red blood cell count 2,695,000, the hemoglobin 56; blood clotting was normal; the blood smear was as on February 11. On April 2, the blood smear was qualitatively erythroleukotic, but the number of immature red cells was scant. On May 2, a blood smear taken about one hour preceding death showed an abundance of early erythroblasts and lymphoid cells, some in mitotic division, and occasional myelocytes.

The weight of the fowl post mortem was 1,200 Gm. Its body was infested with a variety of ectoparasites. The skin and organs were pale; the breast muscle was fairly strong. The abdomen was much distended by clear, yellowish exudate. The liver was grayish brown and weighed 77 Gm.; it was studded with prominent yellowish-gray or yellowish-white nodules, varying in size from 1 to 3 mm. Only the upper third of the right side of the liver was free from such nodules. The spleen weighed from 3 to 5 Gm. and was uniformly pale grayish brown; its consistency was slightly increased. The bone marrow (femur) was pale and grayish brown; its consistency was likewise increased; it contained numerous minute nodules similar to those seen in the liver. The mucosa of the descending loop of the duodenum appeared to be swollen and hemorrhagic; similar areas were found also in the mucosa of the ascending part of the duodenum and in the small intestine.

On microscopic examination, pronounced stasis of lymphoid cells was observed in the liver. The nodules described were formed by myelocytes, many of them in the stage of mitotic division. In the upper part of the right lobe of the liver, which on gross examination appeared to be free from grayish nodules, the microscopic picture was that of erythroleukosis, with only one small myelocytic focus. The hyperplasia of the marrow was extreme, partly (about one-third) myelocytic and partly (about two-thirds) lymphoid. In the other organs, including the spleen, only stasis of lymphoid cells was noted, to a varying degree.

Comment: The qualitative blood picture revealed erythroleukosis at a time when the number of red cells was not appreciably diminished and when the amount of hemoglobin was sufficient. These observations indicate that spontaneous erythroleukosis, like transmitted erythroleukosis, does not develop from preexisting anemia. Temporary improvements in the qualitative blood picture have likewise been observed among the birds inoculated with leukemic material.

*Spontaneous Erythroleukosis with Sarcoma.*—Fowl 515, a barred Plymouth Rock capon, on November 1, appeared ill, showing weakness of the leg. The white blood cell count was 11,000 and the red blood cell count 1,570,000; the hemoglobin was 53. In a blood smear numerous polychrome red cells and polychrome erythroblasts, a few basophil erythroblasts and occasional primitive lymphoid cells were seen (table 3). Three days later the fowl was found dead.

Post mortem the weight was 2,600 Gm.; the breast was fairly well developed; there was much fat in the abdomen and in the subcutaneous tissue; the thymus was small. The heart was studded with grayish, moderately firm, prominent nodules, from 1 to 4 mm. in diameter. About two thirds of the lungs was consolidated, yellowish gray and adherent to the wall of the chest. The liver weighed 103 Gm. and was reddish brown with a slight grayish hue containing four large (from 1 to 2 cm.), and several smaller, grayish nodules. The spleen weighed 7.5 Gm., was soft, red and slightly grayish, and the follicles were prominent. The kidney was soft, brown and slightly grayish. In the left caudal lobe there was a tumor node, about 2.5 cm. in diameter, and a tumor about twice this size infiltrated the perineum. The marrow of the femur and tibia was reddish brown and distinctly grayish, and its consistency was increased.

On microscopic examination some capillaries of the marrow were filled chiefly with lymphoid cells, although not as densely packed as in most cases of erythroleukosis; others appeared to contain normal blood. Granulocytic tissue was scant. In the liver, as well as in the spleen, there was from mild to moderate stasis of lymphoid cells. The microscopic picture of the tumor itself was that of a sarcoma exhibiting great variability of the neoplastic cells from elongated or spindle-shaped forms to the large, round or polygonal type. There was mild, but distinct, stasis in the kidney and adrenal gland. In the latter organ there were also large foci formed by myelocytes and young polymorphonuclear leukocytes.

Comment: The observation of spontaneous erythroleukosis in association with tumor suggests the possibility that erythroleukosis might be secondary to tumor. The data available do not permit any definite conclusion with regard to this possibility. A similar case (that of fowl 675) has been described.

*Atypical Leukemia Associated with Osteosclerosis.*—On Sept. 21, 1929, fowl 441 weighed 1,150 Gm. On October 2, about 0.3 cc. of tar was injected into the marrow of the left femur; the marrow at the same time was partially destroyed. On November 22 the cavity of the right femur was cleaned with a solution of sodium hypochlorite and filled with gutta-percha rods. A blood smear of Feb. 25, 1930, appeared normal. On May 21 (see table 3) the circulating blood appeared to be flooded by white cells, exceeding the red cells in number (the white blood count was 930,000 and the red blood count 560,000). The neoplastic

cells resembled large lymphocytes. Mitotic figures were numerous. There were no transitional forms in any direction to establish the potentiality of these cells. On May 28, the bird was dying and was killed.

At autopsy, the chicken was moderately emaciated, weighing 1,650 Gm. The liver weighed 40.5 Gm. and was uniformly pale grayish brown. The spleen had a similar color and was much enlarged (8.5 Gm.); the follicles were just visible. The left femur was almost entirely ossified; the small amount of marrow that could be obtained was pale grayish. The right femur was likewise ossified, enclosing the inserted gutta-percha sticks. The process of ossification in the tibia was much advanced, but somewhat less complete than that of the femurs. The humerus contained air. The cavity of the ribs was likewise narrowed. The heart was pale grayish and dilated, and measured 3.5 by 5 by 1.7 cm. The parathyroid gland appeared normal. The intestinal tract seemed free from parasites with the exception of one ascaris in the distal part of the duodenum. The cecal follicles were much swollen and reddish on the surface. They measured 1.2 by 0.5 by 0.6 cm.

On microscopic examination, the liver showed pronounced stasis formed by cells closely resembling the lymphoid cells of erythroleukosis, from which they could not be differentiated on morphologic grounds. The character of these cells as seen in smears prepared from this organ was similar to that of the cells seen in erythroleukosis. In the bone marrow there was extreme hyperplasia, formed almost exclusively by similar cells; only a few foci of myelocytes were seen. The microscopic picture of the spleen likewise resembled that seen in severe erythroleukosis, and stasis was marked in the lung, heart muscle and kidney. Attempted transmission to twelve fowls has been unsuccessful.

Comment: The case described is unique. The pathologic picture was that of osteosclerosis with severe erythroleukosis, but the blood picture was dominated by medium and large cells resembling lymphocytes, exceeding the erythrocytes in number. Erythroblasts were absent. Most remarkable was the absence of pronounced basophilia and of nucleoli in most of the leukocytes of the blood. The lymphoid cells that formed the stasis in the liver had, on the other hand, the morphologic character of the early forms of lymphoid erythroblasts.

#### SPONTANEOUS GRAVE ANEMIA UNASSOCIATED WITH ERYTHROLEUKOSIS

A grave anemia of fowls was recently described in England by Bedson and Knight.<sup>4</sup> This anemia is associated with an enormous increase of lipochrome in the circulating blood, causing a yellowish discoloration of the skin and internal organs. The bone marrow shows erythroblastic hyperplasia, but in some instances it is replaced by bone. The mononuclear phagocytes of the spleen and liver are filled with blood pigment. The constant absence of stasis of lymphoid cells in the liver and other organs appears likewise to differentiate this condition from erythroleukosis.

This anemia appears to be chronic and more benign than erythro-leukosis, and its causative agent produces only transient anemia when passed from sick to healthy fowls.

Subsequently Bayon<sup>23</sup> described thirty similar cases, which occurred only in hens of one breed (white Leghorn). All the affected hens were found to harbor a minute cestode, *Davaina proglottina*, in the duodenum. This parasite, Bayon assumed, creates a predisposition to an infection with a hypothetic endocellular microbe ("microplasm"). Osteosclerosis was likewise found by him in several instances. The formation of osteoid tissue was in his opinion a process secondary to the invasion of the marrow.

The contributions of Bedson and Knight and Bayon reached me when the material presented here was about to be reported. It would seem that none of the cases described here as erythroleukosis, except perhaps a few atypical cases mentioned, fall within their definition.

*Case of Grave Anemia.*—A white leghorn hen, no. 235, was received through the courtesy of Dr. Hare on April 10. The hen was well nourished and active, but very pale and yellowish, and its body was full of lice. The examinations of the blood (table 3) showed severe anemia. The condition of the bird seemed unchanged until June 26 when 12 cc. of blood was drawn from the neck for transfer. Death occurred three days later. The bird was very fatty, pale and yellowish. The liver was fragile, yellowish and mottled with small brownish areas. The spleen was brown and about twice the normal size. The marrow of the femur and tibia was pale grayish brown. On microscopic examination, only very slight stasis was found in the liver, which showed large areas of necrosis, diffuse degeneration and small foci of granulocytes (young polymorphonuclear leukocytes and myelocytes). The bone marrow was hyperplastic, but the hyperplasia was chiefly granulocytic and lymphocytic. Transfers of an emulsion of organs to six fowls and of blood to four fowls had negative results.

Comment: Cases such as this have been described by Ellermann as "simple anemia," which he assumed was caused by the leukemic virus and represented an aplastic variety of erythroleukosis. This idea is not well supported. Erythroleukosis seems to be a well defined type of anemia from which cases such as that just described should be separated until their etiologic relationship is proved.

#### EXPERIMENTAL ANEMIAS IN THE FOWL

The numerous studies on the experimental production of anemia of the fowl will not be reviewed because in none of them is the development of erythroleukosis described. It seemed desirable to reinvestigate the possibility of producing erythroleukosis by prolonged bleeding and by the administration of blood poisons.

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23. Bayon, H. P.: Parasitology **21**:339, 1929.



Kaşarinoff<sup>24</sup> did not support his assertion that a leukemic blood picture may be produced in the fowl by the administration of blood poisons by examination of the blood-forming organs, nor has it been confirmed by similar studies of other investigators.

*Anemia Produced by Bleeding.*—After heavy bleedings (from 20 to 50 cc.) there was a transient appearance of polychrome erythrocytes in the circulation. The capacity of the fowl to regenerate erythrocytes on repeated bleeding appears to be so powerful that the attempt to produce erythroleukosis by this means has been abandoned. The possibility that continuous loss of small amounts of blood over a longer period of time would result in erythroleukosis is conceivable, though not probable.

The history of one fowl is briefly given as an example of these investigations.

Fowl 315, on July 25, weighed 1,090 Gm. From November 14 until December 14 a total of 389 cc. of blood was removed in fourteen sessions. The fowl died three days after the last bleeding from an intercurrent purulent infection of the respiratory tract. Preceding the bleedings (July 25) the red blood count was 2,250,000, and three days after the last bleeding it was 2,390,000. The microscopic examination of the femoral marrow, liver and spleen did not show any indication of erythroleukosis.

*Anemia Produced by Injection of Pyrodine.*—Pyrodine (acetylphenylhydrazine) was found effective in producing erythroblastic hyperplasia of the marrow, as well as in promoting the appearance of erythroblasts in the peripheral circulation. Unlike the blood picture in erythroleukosis, that in pyrodine poisoning is dominated by polychrome red cells and maturing erythroblasts. The hyperplasia of the marrow is erythroblastic, with numerous transitional forms to erythrocytes, and not lymphoid as in erythroleukosis. In one case (that of fowl 245) the hyperplasia of the marrow had some resemblance to that in erythroleukosis, but in no instance was stasis of lymphoid cells in the liver or spleen observed. In the capillaries of the liver pigment-laden Kupffer cells are abundant, and similar cells are likewise numerous in the spleen. As an example of this series of experiments the histories of two fowls are given.

On June 22, 1929, fowl 292 weighed 837 Gm. The white blood cell count was 53,000; the red blood cell count, 2,890,000. On June 28 and July 25 the fowl was given ten injections of pyrodine (from 0.5 to 1 cc. of saturated watery solution) subcutaneously. In addition, from November 29 until Jan. 18, 1930, twenty-four intravenous injections of pyrodine were given. The first dose was 2 mg. and was increased subsequently to from 10 to 100 mg. The blood count twenty-four hours after the fourth injection (100 mg. on December 4) was: red blood cells, 835,000; white blood cells, 23,500. In a smear polychrome red cells and erythroblasts were

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24. Kasarinoff: *Folia haemat.* 10:391, 1910.

numerous, but early erythroblasts were scant. Two days later there was an improvement in the blood picture, and 100 mg. of pyrodine was injected, and doses of similar size were administered until death occurred. The extent of rapid regeneration is illustrated by the following figures: December 12, twenty-four hours after injection of 80 mg. of pyrodine, the red blood cell count was 740,000; two days later, it was 1,530,000, and four days later the blood smear appeared normal. On December 31 the red blood cell count was 440,000 and the hemoglobin was 10; on January 14, the red blood cell count was 745,000 and the hemoglobin 14. Death occurred twenty-four hours after the last injection. The fowl was pale and emaciated. The liver was chocolate-brown and very slightly enlarged; the color of the spleen was similar to that of the liver; it was not enlarged. The marrow was reddish brown, soft and juicy. Numerous ascarides were found in the duodenum. In the capillaries of the liver there were numerous brown, pigment-laden Kupffer cells. Similar cells were abundant in the pulp of the spleen. The bone marrow was very hyperplastic; the hyperplasia was mainly erythropoietic, with numerous transitional forms to erythrocytes and to a lesser extent granulocytic. The granulocytes were represented chiefly by young polymorphonuclear leukocytes. Fat was wanting. Leukostasis was not found in the blood-forming organs, the adrenal gland and the heart muscle.

Fowl 245 was killed two days after two intravenous injections of 20 and 40 mg. of pyrodine, given with an interval of twenty-four hours. The blood counts before death were: red blood cells, 1,040,000; white blood cells, 22,500; hemoglobin, 20. Polychrome erythrocytes and erythroblasts were in enormous numbers in the circulating blood, but few basophil erythroblasts were seen. The body was that of an apparently healthy, somewhat fatty fowl. The liver was yellowish with some chocolate-brown hue; it was of normal size. The spleen was purplish red, of about normal size; the follicles were prominent. The bone marrow of the femur was reddish brown, somewhat fatty and interwoven with numerous bony spicules. In the liver and spleen the appearances were somewhat similar to those in the same organs of fowl 292. The pulp of the spleen was studded with pigment-laden cells; the capillary mantle of Schweigger-Seidel, however, which has been maintained to be a part of the "reticulo-endothelial system,"<sup>25</sup> was apparently free. About one half of the marrow was fat, but the remaining part was composed of lymphoid cells similar to those seen in erythroleukosis, with many of them in mitotic division.

The effect of ricin and of tolylenediamine was studied in a similar manner in three fowls each, and of saponin in four other fowls. Erythroleukosis did not result from these procedures.

Comment: It would seem that within a few days the fowl is capable of regenerating its entire volume of blood when it has been gradually destroyed by pyrodine and can repeat the process a number of times before this function is overcome.

The appearance of early erythroblasts in the circulating blood of anemic fowls is not surprising. The blood-forming organs of the lower vertebrates would seem less capable of retaining immature erythrocytes than is the marrow of man, for in the circulating blood of the lower

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25. Staemmler, M.: *Virchows Arch. f. path. Anat.* **255**:585, 1925.

vertebrates multiplication and maturation of erythroblasts may even be physiologic (cf. Freidsohn<sup>26</sup> and Dawson<sup>27</sup>).

Characteristic of the anemia produced by pyrodine in contrast with erythroleukosis is the absence of leukostasis in the organs and the erythroblastic hyperplasia of the marrow with pronounced maturation. This is manifested by the character of the sinusoids, which contain mature erythrocytes in the central parts and erythroblasts near the periphery of the lumen.

#### COMMENT

*Nature of Erythroleukosis.*—The data presented on the transmissible leukosis of fowls are well explained by the assumption of a stem cell common to both erythroblasts and myeloblasts (lymphoid cell of the marrow, according to Pappenheim).<sup>28</sup> This cell in all probability resembles the large lymphocyte (cf. the case of fowl 441), but is not necessarily identical with it. It may mature in either of two directions, depending on the chemical or physicochemical factors created chiefly by their relation to blood channels (Hoogland<sup>29</sup>). Timofejewski and Benewolenskaja<sup>30</sup> incubated lymphoid cells from acute "myeloblastic" leukemia of man and observed their maturation into myelocytes on the one hand and into erythroblasts on the other hand.

The erythroleukosis and myeloid leukosis of fowls appear to consist of a tumor-like growth of immature blood cells deriving from this stem cell, the maturation of which is arrested at an early stage. The regeneration of the blood cells is unlike that seen under physiologic conditions (see Sabin<sup>7</sup>). There is no evidence of a proliferating endothelium, whereas mitosis among the primitive cells is abundant. The frequent observation that the larger blood vessels show no marked increase of lymphoid cells, but that the capillaries are studded with them, points to some factors directed toward a retention of immature cells in the capillary bed.

Ellermann rejected this conception as lacking a basis of observed fact. He assumed that erythroleukosis is similar to pernicious anemia in man, and that both a hypothetic toxin destroys the hemoglobin-containing cells, but spares the "erythrogonies."<sup>31</sup> The data presented

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26. Freidsohn, A.: Arch. f. mikr. Anat. **75**:435, 1910.

27. Dawson, A. B.: Anat. Rec. **45**:177, 1930.

28. The lymphoid cells of the marrow are assumed by Naegeli and by Hirschfeld to be myeloblasts and by Helly to be erythrogonies.

29. Hoogland, H. J. M., in von Heelsbergen, T.: Handbuch der Geflügelkrankheiten, Stuttgart, Ferdinand Enke, 1929, p. 474.

30. Timofejewsky, A. D., and Benewolenskaja, S. W.: Arch. f. exper. Zellforsch. **8**:1, 1929.

31. Helly's term for lymphoid cells with only erythroblastic potencies.

in this report, however, show that, unlike secondary anemia, erythro-leukosis is marked by scant destruction of blood in early cases, and by the appearance of primitive cells in the circulation while there is yet an adequate supply of hemoglobin-containing erythrocytes.

*Diagnosis of Erythroleukosis.*—The diagnosis of erythroleukosis is based on (1) the appearance of early erythroblasts and lymphoid cells in the blood stream, (2) lymphoid cell hyperplasia of the marrow and (3) stasis of lymphoid cells in various organs, particularly in the liver and spleen. Cases of "simple anemia" with polychrome red cells and late erythroblasts in the circulation, but without abnormalities in the blood-forming organs, have not been proved to represent an "aplastic" variety of erythroleukosis, as assumed by Ellermann; their relation to the transmissible agent of the leukemia of fowls requires further investigation.

*Relation of Erythroleukosis to the Anemias of Man.*—Henschen<sup>32</sup> called attention to the similarity of erythroleukosis to the "anemia infantum pseudoleucemia" of von Jaksch. Common to both are the large number of erythroblasts, the enlarged spleen and the dark, but not invariably hopeless, prognosis. Other characteristics of the von Jaksch anemia, such as family occurrence, enormous increase of nucleated red cells after splenectomy, therapeutic failure of splenectomy and liver diet, efficiency of treatment with iron<sup>33</sup> and changes in the bones, have not thus far in the bird been systematically investigated. Extramedullary hematopoiesis seem to occur more readily in anemias of children than in those of adults, and occasionally it may assume a tumor-like growth (Brannan<sup>34</sup>).

Ellermann attempted to coordinate erythroleukosis with pernicious anemia in human beings (cf. also McGowan<sup>3</sup>). In both, maturation of erythrocytes is disturbed at an earlier age than in von Jaksch anemia (cf. Meyer and Heineke<sup>35</sup>). Phagocytosis of erythrocytes may occur in erythroleukosis, but is uncommon (Ellermann<sup>1</sup>). The similarity seems to be the closest to fetal erythroblastosis (von Gierke<sup>36</sup>) and to atypical forms of grave anemia, such as are described by Ferrata and de Negreiros-Rinaldi,<sup>11</sup> in which the mature forms of erythroblasts are absent from the circulation, whereas their basophil precursors appear in considerable amounts.

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32. Henschen, Folke: Arch. f. wissensch. u. prakt. Tierh. **43**:203, 1917.

33. Dr. Amano has failed to observe any change in the course of erythro-leukosis after the administration of liver extract to six fowls.

34. Brannan, D.: Bull. Johns Hopkins Hosp. **41**:104, 1927.

35. Meyer, E., and Heineke, A.: Deutsches Arch. f. klin. Med. **88**:435, 1907.

36. von Gierke, E.: Virchows Arch. f. path. Anat. **275**:330, 1930.

## SUMMARY

Erythroleukosis of the fowl is a disease characterized mainly by (1) severe anemia, with the appearance of lymphoid erythroblasts in the circulation, (2) splenomegaly and (3) the accumulation of cells resembling lymphocytes, presumably precursors of erythrocytes, in the capillaries of the organs, particularly of the bone marrow, liver and spleen.

At the beginning of erythroleukosis, erythroblasts usually appear in the peripheral circulation while there is still an adequate supply of erythrocytes and hemoglobin. In contrast with secondary anemias, the number of polychrome erythrocytes in the circulating blood in proportion to the number of early erythroblasts is not considerable.

Erythroleukosis has been produced by inoculation of blood, organ suspension or Berkefeld filtrate of fowls with myeloid leukosis or erythroleukosis. It occurs spontaneously, but its natural mode of transmission is unknown.

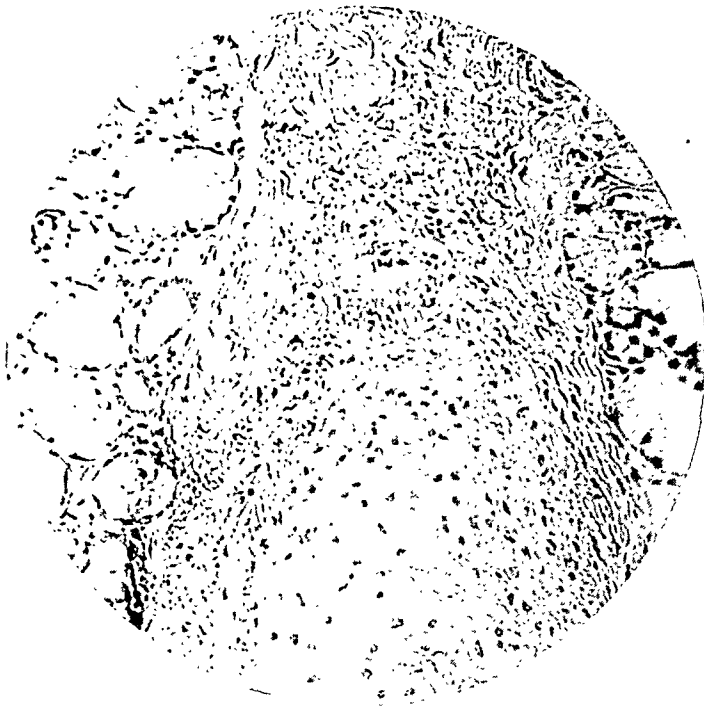
The anemia induced by repeated bleedings or by the administration of pyrodine differs from erythroleukosis mainly by the relatively small number of early erythroblasts in the circulation and by the absence of leukostasis in organs.

# CARTILAGE IN A SALIVARY GLAND\*

H. E. EGGERS, M.D.

OMAHA

In an article on mixed tumors of the palate, the conclusion was reached that the theory of embryonic displacement appeared to be the most probable explanation of these tumors, particularly as compared with the hypothesis that the abnormal elements of connective tissue in



Cartilage in the framework of a salivary gland.

them are derived from glandular epithelium by metaplasia.<sup>1</sup> The specimen reported here, which was received some time after the publication of that article, is of interest in this connection. The material was removed from the mucosa of the pharynx because of local swelling following tonsillectomy in the belief that the enlargement was the result of hypertrophy of the lymphadenoid tissue, a belief which was substantiated by histologic examination. However, in a small portion of underlying salivary gland that formed part of the material there was observed the feature shown in the photomicrograph—a nidus of cartilage located

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\* Submitted for publication, Feb. 7, 1931.

\* From the University of Nebraska College of Medicine.

1. Eggers, H. E.: Mixed Tumors of the Palate, Arch. Path. 6:378, 1928.

in the interlobular connective tissue, entirely unassociated with epithelial elements, even in sections cut at varying depths, and too small even to produce deformity of the connective tissue septum.

It would, of course, be possible to explain the occurrence of this cartilage by the theory of metaplasia, but this would involve the complete disappearance of the original epithelium and the development of the process without the formation of a tumor. It would seem much more probable that the cartilage was the result of inclusion in the connective tissue framework of the gland of a cartilage rest, in this case without the other elements that contribute to the structure of the mixed tumors, and, until the time of its accidental removal, with an absence of the unknown factors that lead to the development of a tumor.

# THE OCCURRENCE OF UNIDENTIFIED SPORANGIA IN THE TONSIL CRYPTS \*

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University of Michigan

ANN ARBOR, MICH.

In about 50,000 tonsils that I have examined during the past thirty-five years, two cases stand out as unique in that the tonsillar crypts contained spherical bodies having hyaline walls and packed full of spores, mostly in an unripe stage. The bodies varied from 30 to 300 microns in diameter. Larger, irregular, partly collapsed or shrunken sporangia containing ripe spores were also present. The histories of the patients, with the diagnoses given at the time, are as follows:

E. B., a woman, aged 21, had frequent sore throat. A tonsillectomy was performed. The pathologic diagnosis was: chronic hyperplastic tonsillitis; increase of stroma; hyperkeratosis; colonies of mouth organisms in the crypts; mold in one crypt, probably *Aspergillus*, tonsillar mycosis.

M. S., a girl, aged 11, had frequent sore throat. A tonsillectomy was performed. The pathologic diagnosis was: chronic hyperplastic tonsillitis; increase of stroma; hyperkeratosis; colonies of mouth organisms in the crypts; abscess of a crypt; numerous colonies of a moldlike organism in the keratohyalin of one crypt, without radiation, but surrounded by a hyaline zone; tonsillar mycosis.

The extreme rarity of this observation has always been a matter of interest to me, and the identification of the organisms in the two cases, apparently identical, an affair of some concern. That the structures were sporangia seemed evident, and this view has been concurred in by all who have seen the preparations. No hyphae were apparent in any of the serial sections made of these tonsils. *Rhinosporidium seeberi* has been the organism lately most frequently considered; and at one time a diagnosis of infection with this organism was tentatively made. Recently, however, a case of infection with *Rhinosporidium seeberi* came into the laboratory, and the study of this case makes it evident that the sporangia in the two cases of tonsillar infection in question are different from those of *Rhinosporidium*. Photographs of slides from the tonsil were submitted for an opinion to Prof. W. H. Taliaferro of the University of Chicago, whose first impression was that they were *Rhinosporidium*, but on my sending a slide to him, he reversed his opinion on the ground that the cytoplasm did not look like that of *Rhinosporidium*, and that the wall around the body did not appear to be the chitinous covering seen in that parasite. Moreover, he thought

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\* Submitted for publication, Oct. 22, 1930.

† Dr. Warthin died on May 23, 1931.



that the hyaline wall around the body showed a certain number of nuclei, suggesting that the wall was laid down by the host and was not part of a parasitic cyst. The absence of nuclei within the parasite he also regarded as evidence that it was not *Rhinosporidium*. Professor Taliaferro showed the slide to Professor Ashworth, during a visit of the latter to Chicago, who agreed that the structures were not *Rhinosporidium*. Dr. C. V. Weller, whose careful and thorough study of a case of infection with *Rhinosporidium* has appeared recently,<sup>1</sup> likewise did not think that these tonsillar parasites were *Rhinosporidium seeberi*, but that they were the



Fig. 1.—Low power view of an abscess of a tonsillar crypt, containing two sporangia with unripe spores and two partly collapsed and emptied ripe sporangia.

sporangia of some closely related organism. I have found nothing in the literature on the tonsil to throw any light on the identity of the organism in question.

As shown in figure 1, the organism consisted of a spherical body with a definite, but narrow, hyaline, nonnucleated capsule, around which was a layer of thin, flattened, squamous epithelium derived from the epithe-

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1. Weller, C. V.: *Rhinosporidium Seeberi*: Pathological Histology and Report of the Third Case from the United States, *Am. J. Path.* 6:721, 1930.

lium of the crypt. This layer contained the nuclei mentioned by Taliaferro, and was derived from the host and was not part of the parasite. It was evident that the structure had developed within the mucosal epithelium. The hyaline capsule was thinner, less hyaline and chitinous than that of *Rhinosporidium sebeci*, but was well defined. It stained pinkish with eosin. Within the capsule was a thickly crowded mass of unripe granular spores, staining bluish with hematoxylin. Only an occasional nucleus could be made out. Two of this unripe form are shown in the figure. In addition there are shown two larger, irregular, partly

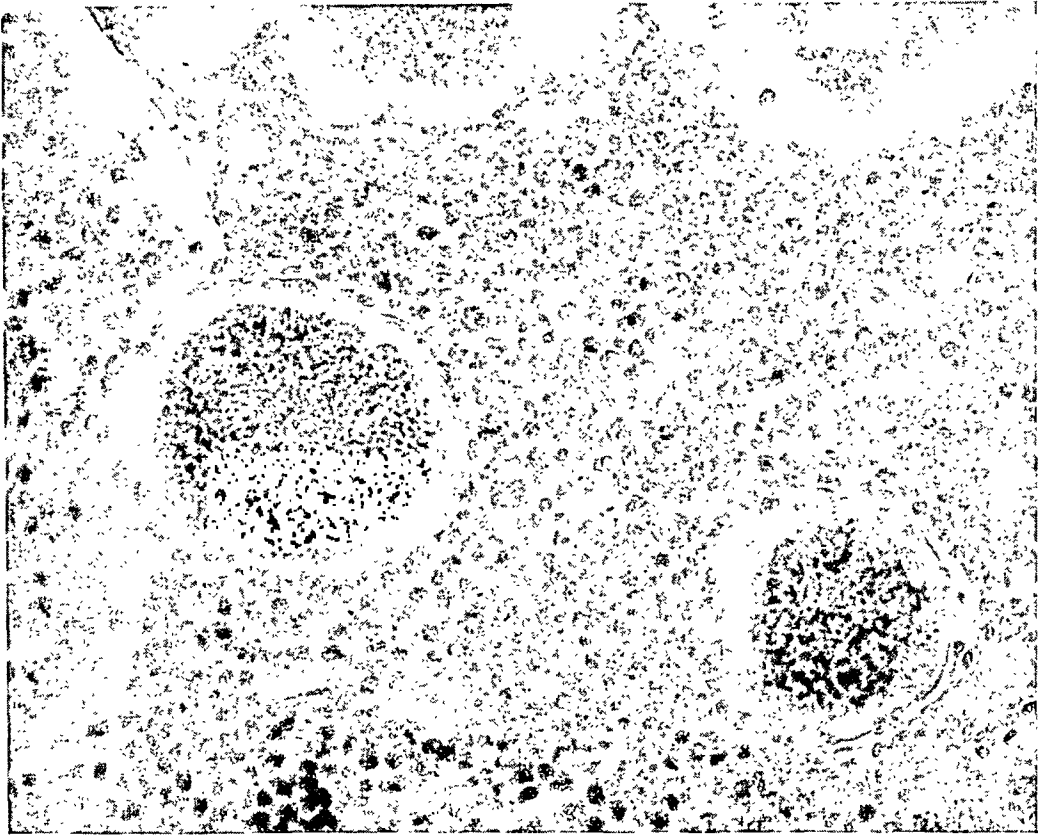


Fig. 2.—Higher power view of the unripe sporangia seen in figure 1.

collapsed sporangia, the spores of which had been in part discharged. Ripe spores and a finely granular material were contained in these collapsed sporangia. The structures were all embedded in the desquamated epithelium, which was heavily infiltrated with polymorphonuclear leukocytes and might have been termed a cryptic abscess. Beyond this there was no evident reaction of the tonsillar tissues to the presence of the parasite. None was found in the lymphoid tissue or stroma. The process was purely a crypt infection.

In figure 2 a view of the two spore-filled sporangia under higher power than in figure 1 is given. The hyaline capsule of the sporangium

with its applied layer of flattened epithelium is well shown. Comparing these sporangia with those of *Rhinosporidium*, the chief difference is in the thinner, less chitinoid capsule of the organism found in my cases. Whether this might be due to the difference in environment between an organism growing within the tissues of a nasal polyp and one growing in the epithelium of the tonsillar crypts cannot be answered. It is also of interest that both my cases occurred in women, whereas *Rhinosporidium seeberi* has been reported as occurring only in male patients.

In conclusion, it is considered important to record the unique observation in the tonsillar crypts of 2 of 50,000 tonsils examined in this laboratory, of sporangia of an unknown parasite, suggesting a close relationship with *Rhinosporidium seeberi*.

# THE RÔLE OF THE ANTERIOR LOBE OF THE PITUITARY GLAND IN GROWTH

WITH SPECIAL REFERENCE TO THE TEETH AND MAXILLAE \*

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NEW HAVEN, CONN.

It is apparent from a survey of literature on the endocrine glands that endocrinologists in general are of the opinion that glands of internal secretion markedly affect the growth, shape, size and placement of the dentition. With the exception of the work of such men as Erdheim,<sup>1</sup> Toyofuku<sup>2</sup> and Gies<sup>3</sup> as regards the effect of the parathyroid gland on the teeth, these opinions are in large part based on empiric and clinical reasoning only.

My own earlier studies<sup>4</sup> of a clinical nature in this connection reveal the fact that variations in form, placement, etc., are largely hereditary. Variations in density and spacing, as well as in the surrounding structure, are very much higher in all groups showing endocrine dyscrasias, but they fail to disclose any regularity in this direction, which would make such dental variations symptomatic of endocrine dyscrasias. The high percentage of variations in these groups, however, made it seem that there was some definite connection between the glands of internal secretion and the growth and development of the teeth. It is felt that perhaps in approaching this from the clinical point of view, one reason for the difficulty in obtaining such regularity was the inadequate symptomatology of endocrine dyscrasias; that is, it is one thing to diagnose a case clinically as of a certain endocrine type but an entirely different thing to know beyond a doubt that a dyscrasia of a particular gland is actually the cause of the symptoms presented.

For this reason, it has been deemed advisable to undertake from the experimental angle a study of this relationship. Owing to its

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\* Submitted for publication, Oct. 3, 1930.

\* From the Yale University School of Medicine.

\* Dr. E. M. K. Geiling supplied me with the earlier extracts used and Dr. E. A. Sharp, of Parke, Davis & Company, those used in the later studies.

1. Erdheim, J.: Zur Kenntnis der parathyreopriven Dentin-Veränderung, Frankfurt. Ztschr. f. Path. **7**:238 and 295, 1911.

2. Toyofuku, T.: Ueber die parathyreoprive Veränderung des Rattenzahnes, Frankfurt. Ztschr. f. Path. **7**:249, 1911.

3. Gies, W. J.: Studies of Internal Secretions in Their Relation to the Development and Condition of the Teeth, J. Am. Dent. A. **5**:527, 1918.

4. Downs, W. G., Jr.: Studies in the Causes of Dental Anomalies, J. Dent. Research **8**:3, 1928.

generally accepted effect on growth, which has been adequately demonstrated clinically and experimentally,<sup>5</sup> the pituitary gland appeared to be the member of the endocrine series most likely to yield definite results in this direction. With that in mind, the following study was undertaken. As such an investigation covered, to a very great extent, uncharted ground, it was necessary to determine first, experimentally, what was the most likely means of influencing such growth, and second, what were the most clearcut methods for determining the rate and the direction of the effect, when obtained. In short, it was necessary to become thoroughly familiar with the field concerned.

Both of these preliminary studies have been done in the present instance in the belief that growth, as applied to any individual organ or member, is only a part of the general process of growth of the organism. The initial stages of the study fell then, naturally, into two divisions: investigations as to the general effects of the hypophysis on growth, and those concerned with measurements of the effects on the teeth and on other organs and tissues.

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5. Cushing, Harvey: *The Pituitary Body and Its Disorders*, Philadelphia, J. B. Lippincott Company, 1912. Dott, N. M.: *The Syndromes of Experimental Pituitary Derangements*, Abst., Eleventh Internat. Cong. Physiol., Edinburgh, 1923. Evans, H. M.: *The Function of the Anterior Hypophysis*, Harvey Lectures, Philadelphia, J. B. Lippincott Company, 1924, p. 212. Evans, H. M., and Simpson, M. E.: Antagonism of Growth and Sex Hormones of the Anterior Hypophysis, *J. A. M. A.* **91**:1338, 1928. Evans, H. M.; Cornish, R. E., and Simpson, M. E.: Potent, Sterile, and Low-Protein Extracts of the Growth Hormones from the Anterior Hypophysis, *Proc. Soc. Exper. Biol. & Med.* **27**:101, 1929. Flower, C. F.; Forkner, C. E.; Kellum, W. E.; Walker, A. T.; Smith, P. E., and Evans, H. M.: Separation of the Principle in the Anterior Hypophysis Affecting Ovulation, from that Controlling Body Growth, *Anat. Rec.* **25**:107, 1923. Korenchevsky, Vladimir, and Dennison, Marjorie, H.: The Influence of the Hypophysis on Metabolism, Growth and Sexual Organs of Male Rats and Rabbits: I. Influence of Extract of Hypophysis on Nitrogen Metabolism, *Biochem. J.* **23**:868, 1929. McLean, A. J.: Transbuccal Approach to the Encephalon, *Ann. Surg.* **88**:985, 1928. Putnam, T. J.: Separation of Growth-Promoting Hormone from That Inducing Premature Oestrus in the Anterior Pituitary Gland, *Arch. Surg.* **18**:1699, 1929. Putnam, T. J.; Teel, H. M., and Benedict, E. B.: The preparation of a Sterile Active Extract from the Anterior Lobe of the Hypophysis, *Am. J. Physiol.* **84**:157, 1928. Putnam, T. J.; Benedict, E. B., and Teel, H. M.: Studies in Acromegaly: VIII. Experimental Canine Acromegaly Produced by Injection of Anterior Lobe Pituitary Extract, *Arch. Surg.* **18**:1708, 1929. Reichert, F. L.: Effects of Anterior Pituitary Extract upon an Hypophysectamized Puppy, *Proc. Soc. Exper. Biol. & Med.* **27**:304, 1929. Smith, P. E.: The Disabilities Caused by Hypophysectomy and Their Repair, *J. A. M. A.* **88**:158, 1927. Teel, H. M.: Diuresis in Dogs from Neutralized Alkaline Extract of the Anterior Hypophysis, *J. A. M. A.* **93**:760, 1929. Teel, H. M., and Watkins, O.: The Effect of Extract Containing the Growth Principle of the Anterior Hypophysis upon the Blood Chemistry of Dogs, *Am. J. Physiol.* **89**:662, 1929. Zondek, B., and Ascheim, S.: Das Hormon des Hypophysenvorder lappens, *Klin. Wchnschr.* **7**:831, 1928.

## EFFECTS ON GENERAL GROWTH

For the investigation of the effects on the general growth, it was felt that the small rodents, particularly mice, lent themselves most readily to the type of study desired, as they were easy to handle in large numbers and their entire life span was sufficiently short to be observable in the laboratory. For this reason, the initial stages of the studies were applied to these animals. Those employed were originally obtained from the excellent colony of Dr. C. C. Little at Ann Arbor, and all of those utilized were the descendants of this original group. In order to eliminate the factor of individual variation as far as possible, the animals were closely inbred throughout the existence of the colony, and as far as was practicable, litter mates were used in the various divisions of the studies involved.

One series of seventy-five mice was divided as follows: (1) a group (twenty-five animals) which was fed the anterior lobe of the beef hypophysis daily; (2) a group (twenty-five animals) which was fed liver in similar quantities daily, and (3) a group receiving the usual diet for these animals. As was the case for the previous studies,<sup>4</sup> in which dogs were used, although this group of investigations was continued for a period of almost six months, the results of this type of treatment were negative. The animals fed liver and those fed hypophysis gained at a slightly higher rate than did the control group. However, as between these two groups, there was practically no variation in increase in size, and it was felt that the only results observed were due to the somewhat higher intake of protein in the experimentally fed groups.

Attention was therefore turned to injection of the extract in the attempt to influence the growth of the animals. My earlier studies in which commercial extracts were used, while productive of some results, had not been sufficiently definite and clearcut to make it seem probable that the best results were to be obtained from this type of experimentation. This was likewise in line with the results of various other experimenters. For this reason it was determined to try a type of extract which could be followed in the laboratory. Evans<sup>6</sup> had very carefully detailed the preparation of an extract which in his hands gave excellent results in influencing the growth of mammalian forms. It was therefore determined to give this particular extract a careful trial. Beginning in October, 1928, I tried this type of extract on several series of animals (mice). The dosage, frequency of medication and other variables were experimented with from every angle. These animals were checked against untreated animals in some cases; in other

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6. Evans (footnote 5, third reference).

cases, the control group received an extract of liver prepared in the same manner as the pituitary extract; and in still other series, the controls received physiologic solution of sodium chloride in similar quantities. In every series of experiments, it was found that the animals receiving the Evans alkaline extract, especially if its reaction was kept well on the alkaline side ( $p_H$  7.6. to  $p_H$  7.8), gained weight at a considerably more rapid rate than did the control littermates.<sup>7</sup> In many instances, this gain in weight was so marked as to appear almost phenomenal.

Quite by accident, as the result of the shipping of some animals that were being experimented on, it was found that if these animals

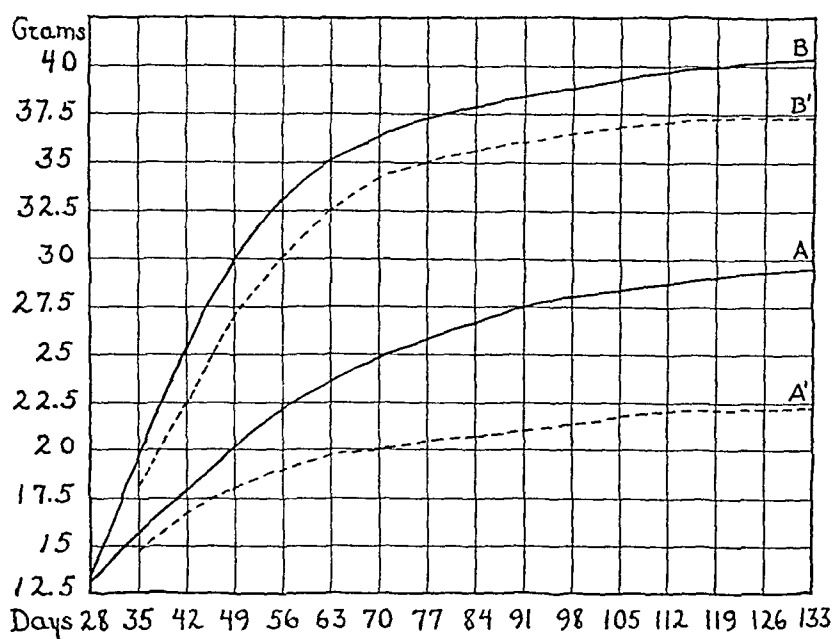


Fig. 1.—Changes in weight: *A*, of untreated controls; *B*, of animals treated by injection of alkaline extract of the anterior lobe of the pituitary gland; *A'* and *B'*, of the same groups, computed on a fat-free basis.

had their food, or particularly their fluid, intake, sharply curtailed, the massive animals that received the extract of the anterior lobe lost weight at a tremendous rate and considerably more rapidly than did the control animals. This was carefully checked, and it was found that a considerable portion of the increase in weight in these animals was due to an increase of water in the tissues, and that the decrease was due to a rapid loss of this water. In order to analyze this, a new group

7. In the later studies, Evans' and Simpson's modifications of the original extract (footnote 5, fourth reference) were in part substituted, and in the later experiments on dogs, in a part of the experiments, an extract made according to Evans' last modification (footnote 5, fifth reference), made by Parke-Davis & Company, was used.

of animals was given similar treatment. Of these, four experimental and four control animals, two of each sex in each instance, were killed, first, at weekly intervals, and later every two weeks, and their bodies thoroughly ground and mixed and chemically analyzed for the presence of water, total nitrogen, total ash and fat, and curves plotted to represent the changes in these contents (figs. 1, 2 and 3).

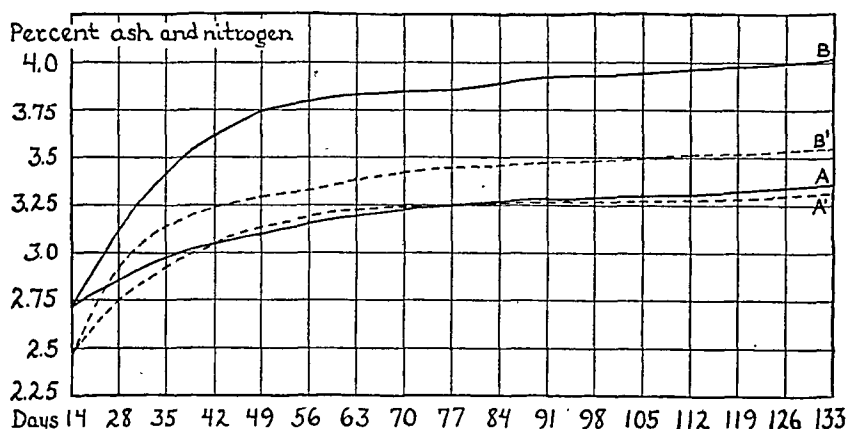


Fig. 2.—Changes in total ash and nitrogen contents ascertained at the periods indicated: *A*, ash content of control group; *B*, of the experimental group; *A*<sup>1</sup>, nitrogen content of control group; *B*<sup>1</sup>, of experimental group.

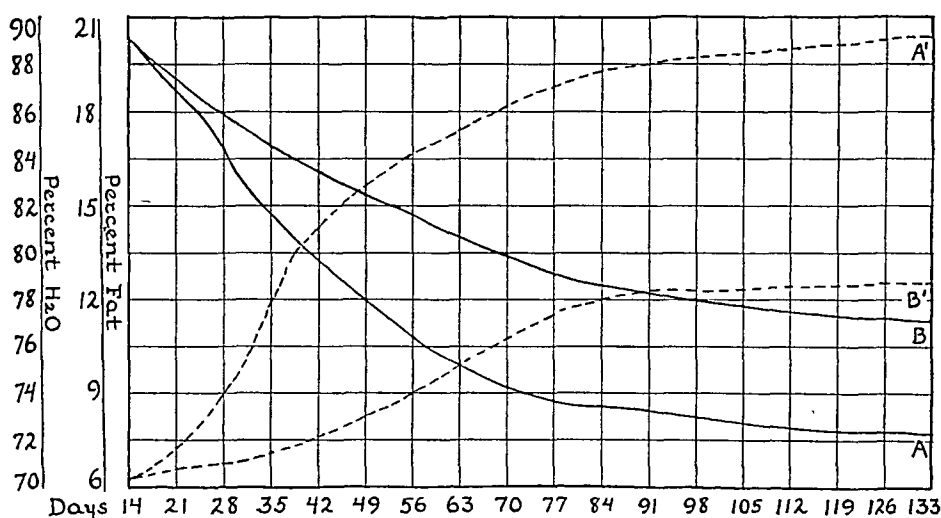


Fig. 3.—Changes in water and fat contents at the ages indicated: *A*, water content of controls; *B*, of animals treated by injections of alkaline extract of the anterior lobe of the pituitary gland; *A*<sup>1</sup>, fat content of controls; *B*<sup>1</sup>, of treated group.

Careful analysis of these results reveals that the animals receiving the alkaline extract of the anterior lobe of the pituitary gland gained in weight at a considerably more rapid rate than did their littermates; that the more rapidly growing animals given injections contained a



considerably higher water content at all levels and a somewhat complementary decrease in fat content; that the more rapidly growing animals given injections had a considerably higher ash content than did the control animals, and that the nitrogen contents of the two groups showed such a slight difference as to be considered negligible.

EFFECTS ON TEETH, LIVER AND OTHER ORGANS

An attempt was made to follow the method of Marshall <sup>7a</sup> in injecting trypan blue or naphthalene brilliant blue or one of the alizarin dyes to obtain definite lines of deposition of calcium in the teeth and to show

TABLE 1.—Measurements of Teeth in Animals Receiving Injections of Extract of Anterior Lobe of Pituitary Gland, Compared with Measurements of Teeth of Untreated Controls

Experimental Animal	Growth, Mm.				Averages
	1st Week	2d Week	3d Week	4th Week	
47	1.2	1.2	1.4	1.3	1.275
51	1.3	1.4	1.4	1.4	1.375
52	1.3	1.3	...	1.3	1.3
54	1.3	1.2	1.3	1.3	1.275
61	1.4	1.4	1.4	1.4	1.4
63	1.3	1.3	1.4	1.3	1.375
92	1.3	1.2	1.3	1.2	1.25
93	1.4	1.3	1.4	1.4	1.375
General average.....					1.328
Control Animal					
101	1.1	1.1	1.0	1.1	1.075
103	1.0	1.0	1.0	0.9	0.975
107	1.2	1.1	1.1	1.1	1.125
116	1.1	1.0	0.9	1.0	1.0
122	1.0	1.0	1.0	1.0	1.0
123	0.9	0.9	0.9	1.0	0.975
133	0.9	1.0	0.8	0.9	0.9
134	1.0	0.9	1.0	0.9	0.95
General average.....					1.000

the rate of growth in this manner. However, the results obtained were not at all satisfactory, and it is felt that no conclusions may be drawn from this portion of the study. On the other hand, by marking the constantly growing incisors with a fine thin disk on a dental mount and marking again at regular intervals, evidences as to differences in rate of growth of these teeth were obtained that were fairly satisfactory. By this method it was determined that the animals treated by injection showed a more rapid growth in the continuously growing incisor teeth than the control animals. In this series it was determined that the average rate of growth of the incisors was about 1 mm. per week in a normal untreated animal. In thirty-eight animals treated by injec-

7a. Marshal, J. S.: An Experimental Study, with Certain Vital Dyes, of the Persistent Teeth of the Albino Rat, J. Dent. Research 2:1, 1920.

tion and studied in this manner not one showed this low rate of growth; the average rate of growth in these animals was 1.3 mm. per week.

The data in the table, giving the actual measurements of one group treated by injection and one group of controls, studied in this way for a period of four weeks, are typical of the rate of growth throughout.

Further than this, roentgenograms of both types of animals demonstrated that the mandibulae and teeth of the animals given injections were larger and of greater density than those of the littermate controls.

It was noted that in these animals there was a marked splach-nomegaly, in which the liver, in particular, was greatly enlarged, even out of proportion to the rate of increased growth. It was likewise apparent that the size of the internal female genitalia was markedly increased in these animals.

In August of 1929, similar studies were begun on dogs, and it was decided to study in addition to the effects of injection of the hypophyseal extract, those of hypophysectomy, on the growth of these animals. In earlier studies the temporal approach to the operation had been attempted with unsatisfactory results, and it was felt that in utilizing very young animals the results to be obtained would be even less desirable. For this reason, the transbuccal approach of McLean,<sup>8</sup> which offered wide access to the region, was decided on, and a number of dogs were the subject of these operations. In all, four successful operations were done, in two animals of each sex, each being about 10 weeks of age. Each of these animals was then grouped with two littermates of the same sex, one of which was handled as an untreated control and one of which received daily injections of the hypophyseal fraction affecting growth. The dosage in these animals was varied, being increased as the animals increased in age and size, until a maximum of 20 cc. daily was attained. Each of the twelve animals in this experiment was studied carefully at the outset, and its physical characteristics and the state of its dentition was noted at that time. The results obtained in each of the groups of three animals were consistent throughout, although varying slightly in individual cases. The dogs receiving the fraction promoting growth grew at a much more rapid rate than did the controls and were, in every sense, larger and heavier. Those that had been hypophysectomized increased in weight and size at a much slower rate than the controls and showed a considerable number of typical stigmas, in particular a myxedematous appearance.

In each case, also, the animals receiving the injections of the extract affecting growth shed their deciduous teeth and erupted the permanent ones at an earlier period than either of the other two types of animals,

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8. McLean (footnote 5, reference 8).

while the hypophysectomized animals were far behind the controls in this respect. Further, the animals given injections had larger maxillae, the hypophysectomized smaller, which gave origin to differences in the position of the teeth (figs. 4 and 5).

One group of males was killed after six weeks of the experimentation, but the observations at autopsy were so slight as to be scarcely more than an indication. After fourteen weeks of the experimentation, one group of females was killed and carefully examined. Unfortunately, on the second day following, owing to a pulmonary infection, it was necessary to kill the second group of females. The results from these autopsies were sufficiently suggestive to indicate the direction of the results. After twenty-two weeks of experimentation,

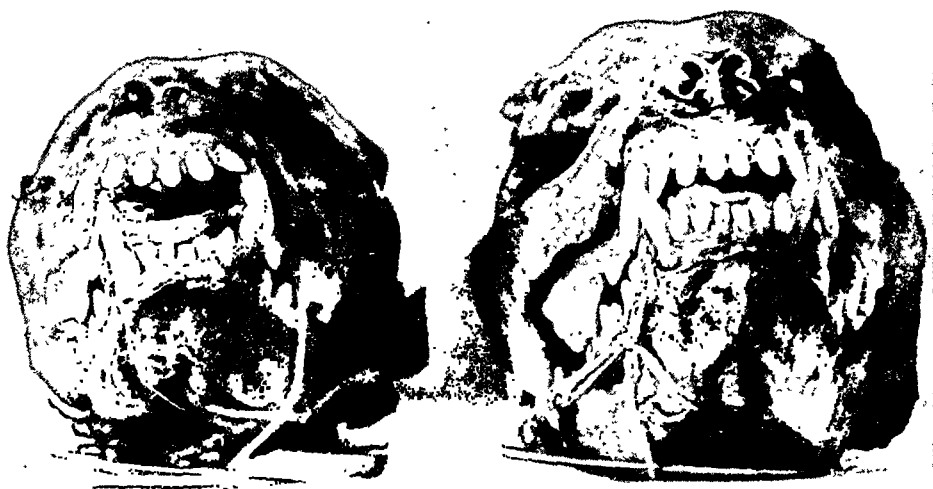


Fig. 4.—Front view (left) of the anterior teeth of a hypophysectomized male dog (no. 10), showing tumbling and “flaring” of the incisors and cuspids, and (right) of a male littermate (no. 11) treated by injection of alkaline extract of the anterior lobe of the pituitary gland, showing the larger maxillae, the spacing and the vertical position of the incisors and cuspids.

the death of one of the animals from advanced toxicosis made it necessary to kill and examine the remaining group. Reference to the accompanying charts will show the differences in rate of growth of the animals involved (fig. 6).

It is noteworthy that the gross changes in these animals were practically identical with those in the earlier series of mice. In each case the injections caused greatly accelerated growth, splanchnomegaly and acceleration in the dentition. In the hypophysectomized dogs, the changes were the opposite of these. Chemical studies on the dogs' tissues were not practicable, but in the case of both types of animals

the animals treated by injection contained, grossly, less fat than did the controls.

Microscopically, the livers of the injected animals showed larger cells, but not sufficiently so to account for the great increase in the gross size of these organs. This was likewise true of the uterus and tubes of the animals. In the case of both organs the reverse was true of the hypophysectomized animals. The uteri of the hypophysectomized



Fig. 5.—Lateral view of the heads of the animals shown in figure 4: above, an animal that had received injections of an alkaline extract of the anterior lobe of the pituitary gland; it shows frontal bulging, a larger head and jaws and a vertical position of the anterior teeth; below, a hypophysectomized animal, showing frontal flattening, smaller head and jaws and flaring of the anterior teeth due to a diminished arch.

animals were very infantile microscopically, as well as grossly, while those of the controls and of the animals treated by injection showed a marked increase in the endometrial glands and epithelium. Likewise, the ovaries of the hypophysectomized animals showed no evidence of advanced follicles, while those of the controls and of the animals treated

with the extract promoting growth showed many far advanced, but not ripe, follicles. The animals given injections showed a marked increase in stroma, over the controls, in each of these organs. Microscopic study of the teeth and investing tissues yielded negative results in each instance. No changes were to be observed in pulpal cells, ameloblasts, dentin, enamel or surrounding tissues. Neither were there differences in size between corresponding teeth in the different groups of these animals.

Throughout the lymphogenous organs, there was a noticeable increase in the size of the organs in the animals given injections, and microscopically this was found not to be due to an increase in the lymphocytes,

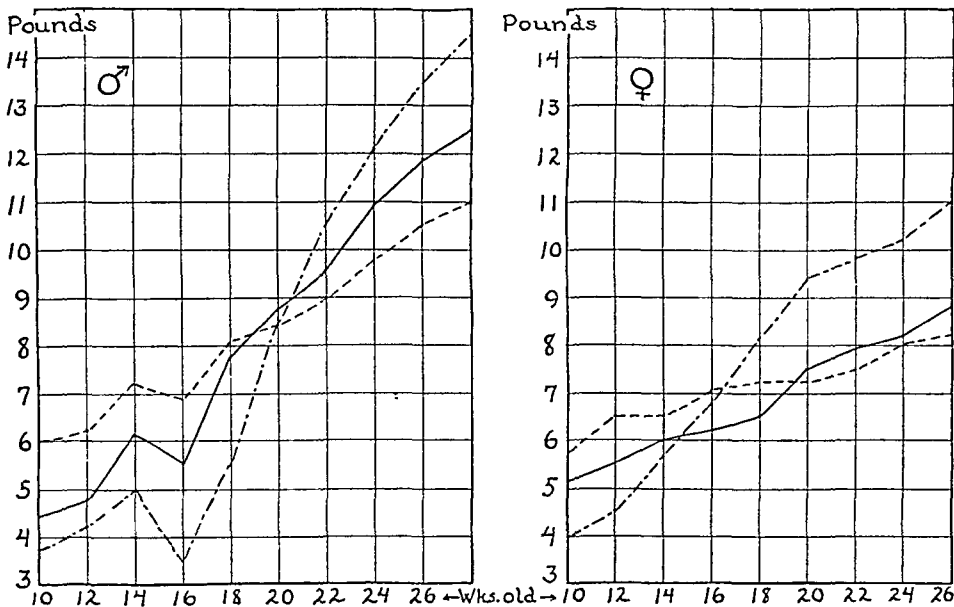


Fig. 6.—The chart at the left shows the growth curves of a litter of male dogs, carried under experimental conditions for twenty-four weeks; that at the right, the growth curves of a litter of female dogs, carried under experimental conditions for sixteen weeks.

but rather to a marked increase in the sustentacular tissue and to an edematous change in the organs. The liver cells showed necrosis, especially in the region of the central veins. This, with the microscopic changes in the kidneys and those in the lymphogenous organs, suggested the presence of some toxic change. What the cause of this change was, it would be difficult to determine. The method of preparation of the extract is one possibility. More likely, however, is the possibility that such greatly accelerated metabolism was itself productive of a toxicosis. Throughout all of the organs examined there was a varying apparent increase in the connective tissue elements in the animals

treated by injection, most marked in the lymphogenous organs, that is, the lymph glands, thymus and spleen. No change of this sort was noted in the salivary glands, pancreas or lungs, but a less marked one was present in the liver and kidneys.

Roentgenograms of the mandibulae of the mice showed an increase in size and density of these and of the continuously growing incisors in the animals given injections. There was likewise an apparent change in the dentition and the mandibulae of the dogs. In addition to this, in the dogs there was a marked difference in the size and shape of the head, especially noticeable in the frontal region (figs. 4 and 5).

#### CONCLUSIONS

There is in the anterior lobe of the hypophysis a substance that markedly affects the general growth of the organism. This effect is exercised in the direction of maintaining the water content of the tissues of the animal at a high level and in reducing the animal's tendency to put on fat. Its tendency is to increase the total mineral content of the tissues over a normal level for a given period of age. Its effect on the dentition of the animal is to markedly accelerate this process and to change the position of the teeth in the jaws, owing to a difference in the size of the jaws. Further, there is a probable toxic effect, evidenced microscopically in the liver, kidney and lymphogenic organs and due probably to the toxic products of too rapid stimulation of metabolic processes. There is likewise an apparent tendency for the growth-promoting hormone to stimulate especially the growth of the supporting tissue elements.

If these results are compared with those of Donaldson<sup>9</sup> and others, on the normal growth of rodents, the conclusion seems warranted that the effect of the extract of the anterior lobe of the hypophysis is to stimulate these animals in the early period of rapid growth (the third phase of Donaldson) to an even greater rate of growth and to somewhat prolong this period into the later, or fourth, phase. It also seems apparent that these effects are in no sense specific for the dental mechanism or inclined to be productive of specific anomalies of the teeth, but are exercised simply in the direction of rate and time of development, and that if there is a specific effect involved, it is in the direction of further accelerating the formation of connective tissue, which is already being formed at a rapid rate at this period, and of increasing the fluid content of these tissues.

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9. Donaldson, H. H.: A Comparison of the White Rat with Man in Respect to Growth of the Entire Body, Boas Anniversary Volume, New York, G. E. Stechert & Company, 1906.

It seems apparent from these and earlier studies that the eruption and growth of the teeth and of their investing tissues may be considered only as a part of the general phenomena of growth of the body, and may be influenced by the pituitary gland in a specific direction only insofar as the time and velocity of development of the various tissues and organs may be influenced.

# THE ENDOCARDITIC PROCESS IN CHILDHOOD\*

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The rarity of endocarditis during the first years of life has been remarked on by various writers in the past. Thus, Holt found no case of endocarditis in the course of 1,000 autopsies on infants under 3 years of age, and Hochsinger confirmed the extreme rarity of the condition in persons less than 5 years of age, as compared with its great frequency in those between the ages of 10 and 14. On the other hand, clinicians claim a relatively high percentage for valvular lesions occurring during the first ten years of life. Thus Guttman (1893) reported that 5 per cent of cases of valvular disease occurred during the first ten years, 18 per cent between 10 and 18 years of age, and from 22 to 23 per cent after 20 years of age. More precisely G. Schultze (1906), working on a vast material, obtained the following data: 4.29 per cent of cases occurred from birth to 10 years, 36.60 per cent (the maximum percentage) from 10 to 20 years and 28.27 per cent from 20 to 30 years. After 30 years of age, the percentage becomes steadily lower.<sup>1</sup>

From these statistics it appears that valvular lesions occur with some frequency during the first years of life, referable in part at least to an unrecognized endocarditic process.

## INCIDENCE OF VALVULAR ENDOCARDITIS IN CHILDREN, AS NOTED IN AUTOPSY REPORTS

I have tried to determine, as exactly as possible, the incidence of valvular endocarditis in children, and have made use of the postmortem material of the Institute of Pathological Anatomy of Florence, amounting to 4,952 autopsies. The statistics of age and sex are shown in figure 1. In all, 179 cases of endocarditis were noted, 93 of which were acute (verrucose, vegetative and ulcerative) and 83 subacute or recurrent. Figure 2 shows the relative frequency of the condition at different age periods and of itself is a demonstration of the rarity of anatomic evidences of endocarditis in childhood. The rarity becomes

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\* Submitted for publication, Dec. 5, 1930.

1. Jores, A., in Brüning, H., and Schwalbe, E.: *Handbuch der allgemeinen Pathologie und pathologischen Anatomie des Kindesalters*, München, J. F. Bergmann, 1921.



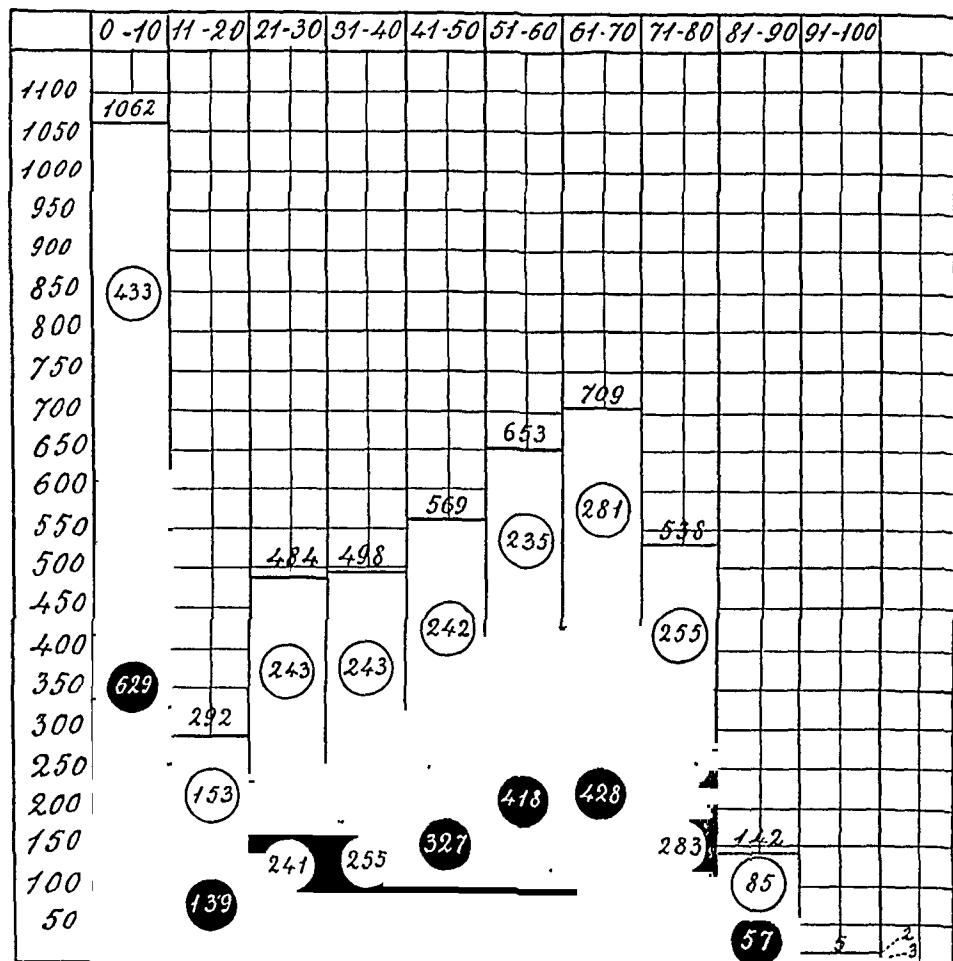


Fig. 1.—Distribution of the autopsy material according to age and sex. The solid black columns represent the male sex; the white columns, the female sex.

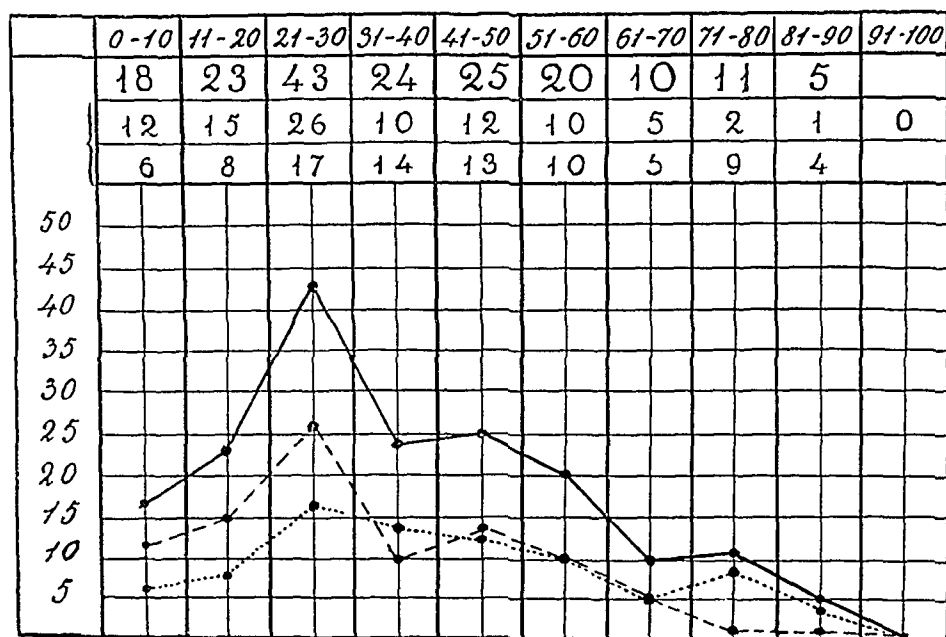


Fig. 2.—Distribution of the cases of endocarditis by age periods. The continuous line represents all the cases; the broken line, the acute cases, and the dotted line, the chronic or recurrent cases.

still more striking when the figures are reduced to percentages of the total autopsies in each age period, as in figure 3.

It is clear that during the first ten years of life valvular lesions of the endocardium are rarely found (18 cases among the 1,062 autopsies of that age period, i. e., 1.69 per cent). This rarity is still more striking for the first year of life, for of the 18 cases in the first decennium, only 2 occurred in infants under 1 year of age, 6 in children from 1 to 5, and 10 in children from 6 to 10 years of age.

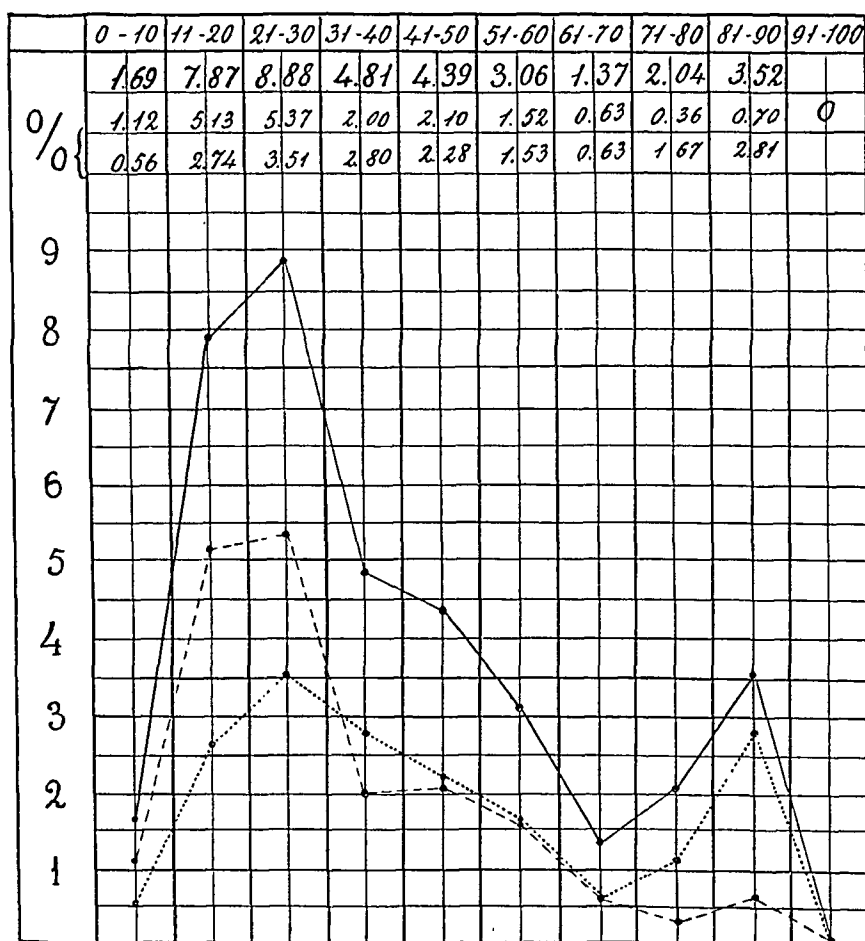


Fig. 3.—Percentage of cases of endocarditis in the total number of autopsies of each age period.

*Interpretation of Apparent Infrequency.*—In explanation of this rarity of valvular disease in infancy, Finkelstein assumed a special immunity of the endocardium of sucklings against inflammatory agents, severe and numerous though such agents are in this period. The real explanation is probably much simpler. The postmortem diagnosis of endocarditis, both in children and in adults, is usually based on macroscopic evidence of thrombotic vegetations on the valves or on the parietal endocardium. Macroscopic evidence of this kind is, however, cer-

tainly incomplete and fallacious. In persons dying of toxic infectious diseases, valves appearing perfectly healthy to the naked eye show microscopic changes, which are evidently inflammatory, even without the presence of any thrombotic deposits (Baldasjari,<sup>2</sup> de Vecchi,<sup>3</sup> Czirer<sup>4</sup>).

The diagnosis of endocarditis is therefore not always possible without a histologic examination, and ought to be extended to all cases in which any active participation of the valvular tissues can be demonstrated microscopically. Moreover, as I have maintained elsewhere,<sup>3</sup> the valvular changes are to be regarded as the characteristic feature of the process, while the thrombotic lesions must be considered as merely secondary changes, often absent throughout the course of the disease. The necessity of a histologic basis for the diagnosis of incipient valvular lesions, which I have long maintained, has received very tardy acknowledgment. Only recently Holsti<sup>5</sup> took up the idea and confirmed the great value of microscopic research in the diagnosis and interpretation of endocardial lesions that would otherwise escape detection.

These general considerations regarding the anatomic diagnosis of endocarditis are still further justified in the case of endocarditis in infants, in whom the valvular apparatus is more delicate and the initial changes in it more difficult to detect. Moreover, it is probable that before the third year of life (as in the embryo) the constitution of the blood (morphologically an erythropenia, chemically a deficiency of globulin) does not lend itself to the formation of thrombi. Hence there is an insufficiency of the elements necessary for the formation of endocardial vegetations.

#### MATERIAL USED FOR MICROSCOPIC STUDY

The present investigations have been directed toward a systematic study of these initial stages of endocarditis, as demonstrable histologically even when macroscopic examination does not suggest their presence.

The valves of the infant's heart lend themselves readily to such a study of the early phases. One is dealing with conditions almost experimental, because the disease is developing in tissues not previously altered. Naturally, the lesions in such cases are not widespread or marked. If they were, they would be evident to the naked eye. One deals always with incipient and circumscribed lesions; hence this material is useful in the interpretation of the pathogenesis of processes

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2. Baldasjari: Tesi di Laurea, Bologna, 1907; *Centralbl. f. allg. Path. u. path. Anat.* **20**:97, 1909.

3. de Vecchi, B.: *Arch. di pat. e clin. med.* **4**:3, 1926.

4. Czirer, L.: *Virchows Arch. f. path. Anat.* **213**:272, 1913.

5. Holsti, O.: *Arb. a. d. path. Inst. zu Helsingfors* **51**:401, 1928.

*Cases Examined*

Case	Autopsy	Age	Sex	Anatomic Diagnosis
1	16523	1 mo.	F	Intestinal malformations; volvulus; laparotomy, purulent peritonitis
2	301;N.S.	2 mo.	M	Tuberculous mastoiditis; miliary tuberculosis; meningitis
3	15064	3 mo.	M	Status thymolympathicus; confluent bilateral bronchopneumonia
4	14718	3 mo.	F	Suppurative cerebrospinal leptomeningitis; abscess of brain
5	15054	4 mo.	M	Hyperplasia of Peyer's patches; multiple subcutaneous abscesses
6	15046	4 mo.	F	Diphtheritic laryngitis; bilateral bronchopneumonia
7	16429	6 mo.	F	Bilateral confluent bronchopneumonia
8	16428	7 mo.	M	Bronchopneumonia on right side
9	16496	7 mo.	F	Metapneumonic empyema on left side
10	15045	8 mo.	M	Bilateral bronchopneumonia; hemorrhagic pulmonary infarcts; softening; purulent pleurisy on left side; parenchymatous degeneration
11	14762	8 mo.	M	Bilateral bronchopneumonia; empyema on right side; acute nephritis; splenic tumor
12	14820	8 mo.	M	Suppurative leptomeningitis of vault
13	14763	9 mo.	M	Bilateral bronchopneumonia; rickets; hypoplasia of right kidney; parenchymatous degeneration
14	16432	9 mo.	F	Bronchopneumonia on right side
15	16074	10 mo.	M	Acute enteritis; nasal impetigo; parenchymatous degeneration
16	16420	12 mo.	M	Bilateral bronchopneumonia
17	14554	12 mo.	M	Hyperplasia of Peyer's patches; splenic tumor; mesenteric lymphadenitis; bilateral pleurisy; serofibrinous pericarditis; septic infarcts of lung; acute nephritis
18	16416	13 mo.	M	Bilateral bronchopneumonia; pericarditis; bilateral fibrinous pleurisy
19	16418	13 mo.	M	Acinodose tuberculosis of both lungs; nodular tuberculosis of spleen and liver
20	180;N.S.	14 mo.	M	Epidemic cerebrospinal leptomeningitis
21	16519	16 mo.	M	Bilateral bronchopneumonia; thrombosis of suprahepatic vein; ascites; splenomegaly
22	14819	16 mo.	M	Bilateral bronchopneumonia; bilateral fibrinous pleurisy; suppurative leptomeningitis
23	16732	20 mo.	M	Typhoid enteritis; suppurative otitis media on left side
24	16755	21 mo.	F	Old tonsilar phlegmon; bilateral bronchopneumonia; chronic mitral endocarditis; multiple cerebral softening; acute cholecystitis; splenomegaly
25	16417	2 yr.	F	Bilateral bronchopneumonia; pertussis
26	16412	2 yr.	M	Bilateral bronchopneumonia; suppurative pleurisy on right side; pleurectomy
27	16502	2 yr.	F	Unresolved pneumonia; bilateral suppurative pleurisy; parietal cardiac thrombosis
28	15074	3½ yr.	F	Postdiphtheritic paralysis; bilateral bronchopneumonia; endocarditis verrucosa mitralica acuta
29	16734	3½ yr.	F	Necrotic angina; septicemia; macular facial erythema
30	14680	3½ yr.	F	Bronchopneumonia on right side; status thymolympathicus
31	16426	4½ yr.	M	Osteomyelitis of right tibia; fibrinous pericarditis; multiple pulmonary infarcts
32	16486	5 yr.	M	Acute pharyngolaryngotracheal inflammation; suppurative otitis media
33	16733	5 yr.	M	Necrotic scarlatinal angina; bed sores; acute nephritis
34	14654	5 yr.	M	Descending diphtheria; follicular enteritis; induration of the aortic margin of the mitral valve
35	16360	5 yr.	F	Descending diphtheria; acute bronchitis; focal pulmonary atelectasis; meningo-encephalic congestion
36	16507	6 yr.	M	Purulent arthritis of left knee; empyema on right side; multiple pulmonary infarcts
37	16430	7 yr.	F	Mastoidectomy on right side; acute serous leptomeningitis
38	16147	7 yr.	F	Suppurative osteomyelitis of petrous portion of temporal bone; multiple pulmonary abscesses; hematogenous suppurative nephritis; verrucose endocarditis of mitral valve
39	16431	8 yr.	M	Necrotic appendicitis; localized fibrinous peritonitis; chronic pulmonary tuberculosis
40	141;M.S.	9 yr.	M	Measles; bronchopneumonia on right side
41	16499	14 yr.	M	Bilateral bronchopneumonia; diplococcal sepsis
42	16491	16 yr.	F	Bilateral pulmonary tuberculosis (ulcerocaseous)

that usually are studied only at a much more advanced, more complex stage.

The material that I have used for my own observations comprises forty-two cases, selected from autopsies performed during the past few years. I have not considered it necessary to extend these observations over a longer period, because, as will be seen, the results are sufficiently clear and consistent.

As regards valves presumably affected, particularly as regards experimental results,<sup>6</sup> I must refer to considerations set forth elsewhere, which explain the difficulty of basing the observations on a large amount of material. It is essential that the suspected valve be subjected to thorough examination, and not to a superficial, hurried search. Valves that when first seen under the microscope appear unaffected show obvious lesions in other sections. In order to make an exact diagnosis, it is therefore necessary to search through the whole valvular apparatus, sometimes in serial sections, under a suitable magnification, for only so is it possible to detect lesions that might otherwise escape notice. All this requires system, time and patience; therefore, the cases studied cannot be very numerous.

From the anatomic diagnosis it is seen that only four cases (24, 28, 34 and 38) showed macroscopic changes in the valves. In two of these (28 and 38) there were the lesions of recent verrucose endocarditis; in the other two (24 and 34) the lesions were those of chronic sclerosing endocarditis. I might have included other cases in the list, as occurring in infancy, but have restricted myself to these four cases for comparison with those in which there was no visible lesion in the valves.

The material is subdivided into eight groups on a nosologic basis. This grouping proves useful for the demonstration of the histologic results.

#### HISTOLOGIC OBSERVATIONS

*Group 1.*—Group 1 included five cases (5, 23, 31, 36 and 38) of suppurative processes in various parts of the body (osteomyelitis, arthritis, otitis complicating typhoid fever, cutaneous abscesses, etc.), in which there was very probably a septic process at work.

The valves of the heart in these cases always showed histologic lesions, varying in severity and extent. As would be expected, the most intense lesions occurred in case 38, in which the valvular lesions were already visible macroscopically on the mitral segments. In an extensive area on the atrial surface of the mitral valve, there was loss of endothelium with hyalinization and necrosis of the adjoining valvular tissue. The desquamated endothelium was largely enclosed in a

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6. de Vecchi, B.: *Le malattie del cuore*, Rome, 1923, vol. 7, p. 72.

fibrinous network. There were numerous cells of lymphocytic type in the deeper layers of the valve, surrounding the regressive lesions in the valvular tissues. In the subendothelial layers nearest the desquamated area there were large cells, each with abundant cytoplasm and a vesicular nucleus. These were easily interpretable as histiocytes (fig. 4 *A*). In other parts of the valve, even where the endothelium was apparently healthy, evidences of histiocytic reaction were still to be met with, and this not only in the superficial subendocardial layers, but also extending into the deepest layers of the valvular tissue, and sometimes accompanied by an abundant and diffuse lymphocytic infiltration. Evidence of hyaline thrombosis following the desquamation of endothelium was found in some sections. In some parts one might also see deposits of fibrin, enmeshing erythrocytes and leukocytes. A migration of elements originating in the underlying tissues into the initial thrombus could not, however, be observed. The thrombus was a passive phenomenon occurring subsequent to the formation of the lesions established on the valve.

That lesions of this type were present in areas macroscopically healthy was proved by the fact that a histologic examination of the apparently healthy aortic valve in the same case showed areas of endothelial desquamation and edema with detachment of the underlying valvular layers, a somewhat diffuse infiltration by lymphocytes and the presence of histiocytic elements both in the superficial and in the deeper layers. The type of lesion was the same; the difference was in the intensity of the pathologic change and above all in the absence of the secondary phenomenon of superficial thrombosis.

Similar phenomena were evident in the other cases also: limited endothelial desquamation, edema and hyalinization of the valvular layers, lymphocytic infiltration and histiocytic reaction. The latter was marked, especially at the points of attachment of the valves, and was not necessarily accompanied by desquamation of the endothelium. In case 23 (purulent otitis complicating typhoid enteritis) the lesions were marked and particularly extensive, the histiocytic reaction extending down into layers far distant from the endothelial lesion.

*Group 2.*—Group 2 included four cases: one following scarlet fever (33); two of acute type, and followed probably by septicemia (29 and 32), and the last (24) with healed anginal lesions, but with bronchopneumonia, splenomegaly and septicemia, thickening of the edges with thrombotic deposits on the mitral valve, and, as a consequence of the detachment of the latter, the formation of multiple foci of cerebral softening.

As was to be expected, histologic examination in case 24 showed very serious lesions on the mitral valve. These consisted in an intense, widely spread edema, with hyalinization of the valvular layers, accom-

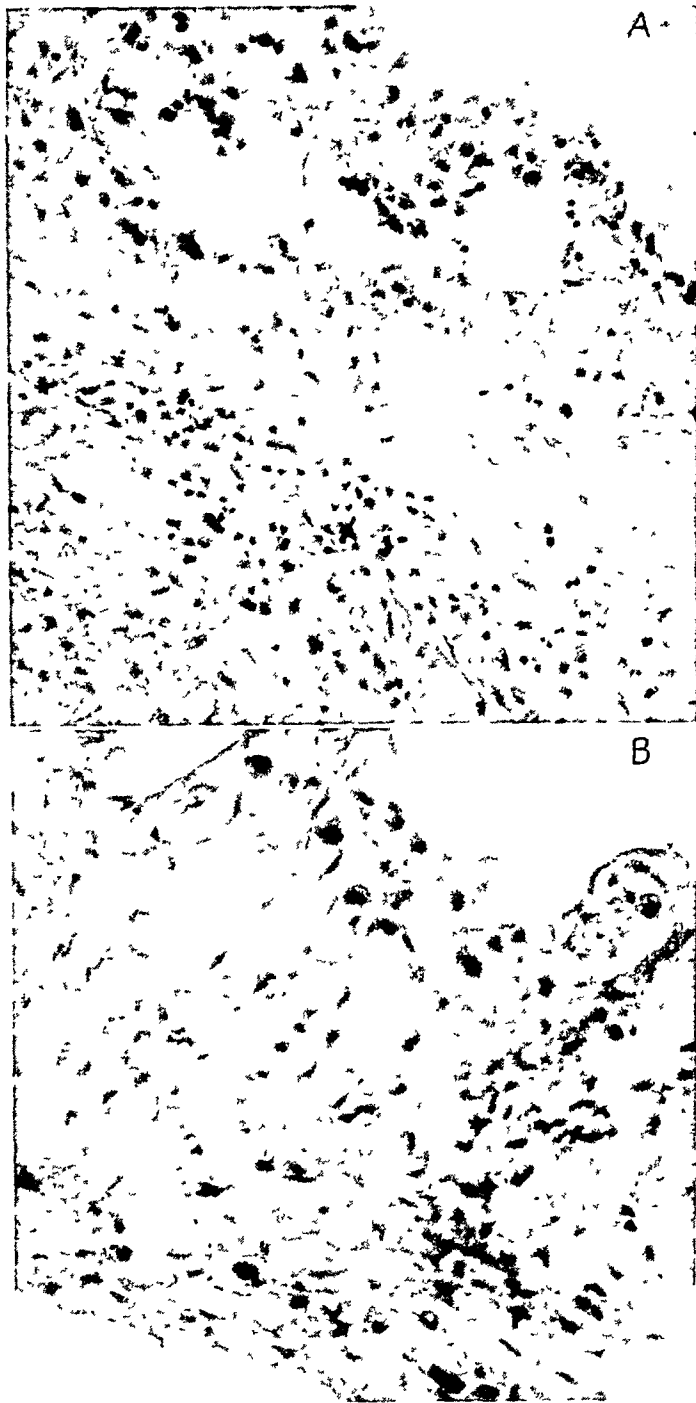


Fig. 4.—*A*, mitral valve in case 38, that of a boy, aged 7, with suppurative osteomyelitis in petrous bone, abscesses of the lung, suppurative nephritis and verrucose mitral endocarditis. The initial stage of the latter was visible macroscopically. The photomicrograph shows desquamation of endothelium and agglutination of the exfoliated cells in a fibrinous network, hyalinization and partial necrosis of the valvular tissue, and lymphocytic infiltration and deep histiocytic reaction delimiting the degenerative necrotic zone.

*B*, Aortic valve in case 24, that of a girl, aged 21 months, with tonsillar phlegmon, cholecystitis, multiple cerebral softening and verrucose endocarditis of the mitral valve. The latter was visible macroscopically. The aortic valve was macroscopically intact. The photomicrograph shows desquamation of the endothelium, edema and histiocytic reaction of both surfaces of the valve.

panied by an intense histiocytic reaction, which passed into the deeper structure, but was less marked near the surface. In the same case identical and equally extensive lesions were seen on both the ventricular and the arterial surfaces of the aortic valve, although this appeared normal to the naked eye (fig. 4 *B*).

A marked superficial and deep histiocytic reaction, accompanied by a less marked lymphocytic infiltration, appeared in case 29, both in the mitral and in the aortic valve. A typical focus of endocarditis, with extensive desquamation of the endothelium and agglutination thereof in a fibrinous network, with intense lymphocytic infiltration and peripheral histiocytic reaction, had developed in the aortic valve in case 32 (fig. 5 *A*); in this case, too, the valves appeared normal to the naked eye. Valvular lesions, but much milder and more circumscribed, were also present in a case of scarlatinal angina (33).

*Group 3.*—Four cases of diphtheritic infection were studied. Of these, three were of acute type (6, 34 and 35), and one (28) had run a more chronic course, death being due to postdiphtheritic paralysis. In case 28 the autopsy showed acute verrucose endocarditis of the mitral valve, while in case 34 there was evidence of induration of the aortic segment of the mitral valve.

The tissues obtained in case 28 were insufficiently fixed, so that a proper study of them could not be made. In case 34, lesions of the infiltrative and histiocytic reaction type were present not only in the mitral valve, but also in the aortic (fig. 5 *B*), though the latter was macroscopically normal.

In case 6 (diphtheria in a suckling) the mitral valve showed a few foci of lymphocytic infiltration and histiocytic reaction in the valvular layers, with partial desquamation of the endothelium. But of special importance were the histologic lesions in case 35, in which the valves had appeared healthy at the autopsy. Both the mitral and the aortic valves showed areas of endothelial desquamation, with extensive hyalinization and incipient necrosis of the underlying valvular layers, also slight lymphocytic infiltration and histiocytic reaction, not only in the superficial subendothelial structure, but also in the deep layers, especially in the border area between the degenerative necrotic, and the healthy, valvular tissue (fig. 6 *A*).

*Group 4.*—There were four cases of suppurative leptomeningitis, two being probably primary epidemic (4 and 20) and two secondary (12 and 37). Focal lesions were demonstrated histologically in the valves in three cases, especially in the mitral valve in case 12 (edema of the valvular layers, deep histiocytic reaction). Only in case 37 did the valves show no histologic lesion.



*Group 5.*—Group 5 included two cases of acute exudative peritonitis: one (1) in a new-born infant, secondary to operative interference, and the other (39) in a child with tuberculosis following necrotic appendicitis. Infiltrative and reactive lesions of the valvular

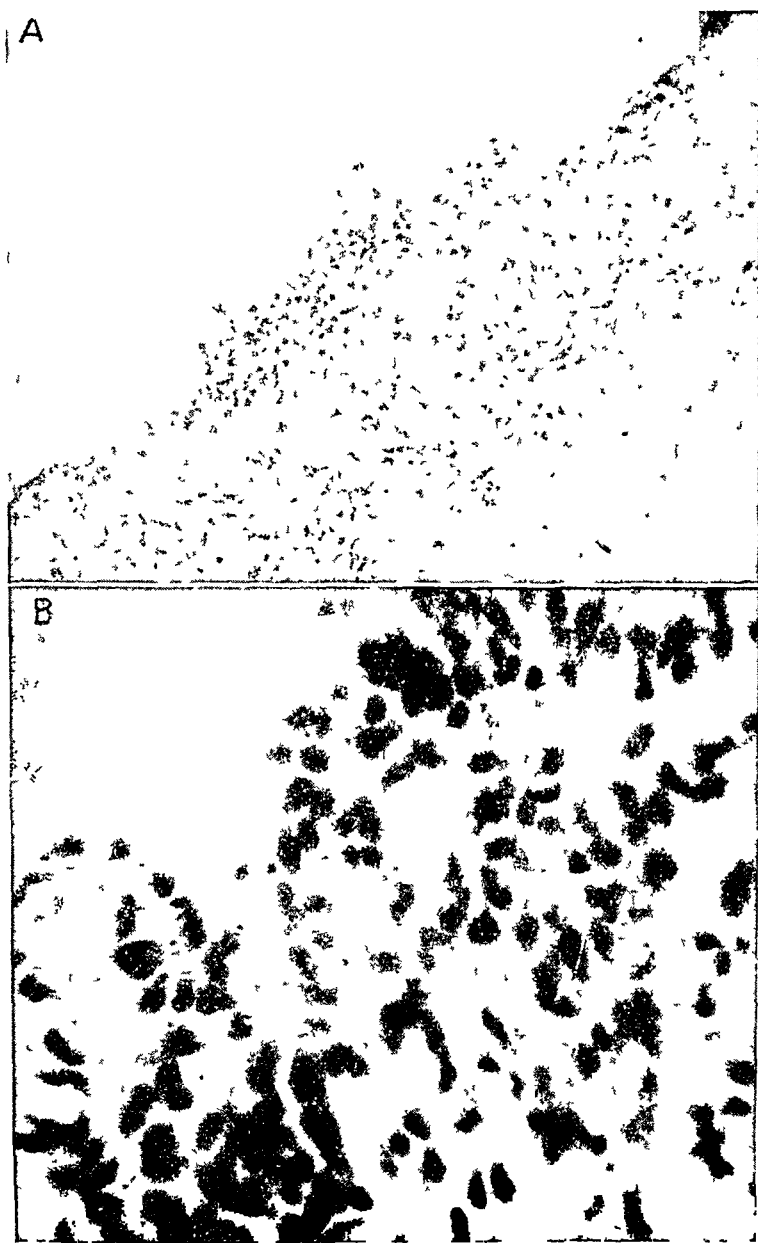


Fig 5.—*A*, aortic valve in case 32, that of a boy, aged 5, with acute pharyngolaryngitis and suppurative otitis. The valve was macroscopically healthy. A typical focus of commencing endocarditis is shown.

*B*, aortic valve in case 34, that of a boy, aged 5, who died from descending diphtheria. Thickening of the mitral valve was found. The aortic valve was macroscopically normal. Note lymphocytic infiltration and diffuse deep histiocytic reaction.

tissue were found in both cases, but they were of particular intensity in case 1, in which the infiltrative and histiocytic reactions were most marked in the deeper layers of the valve; that is, at a point distant from its free margins.

*Group 6.*—Two cases of enteritis comprised group 6: one was acute (15); the other was more chronic and complicated by pleuropericarditis

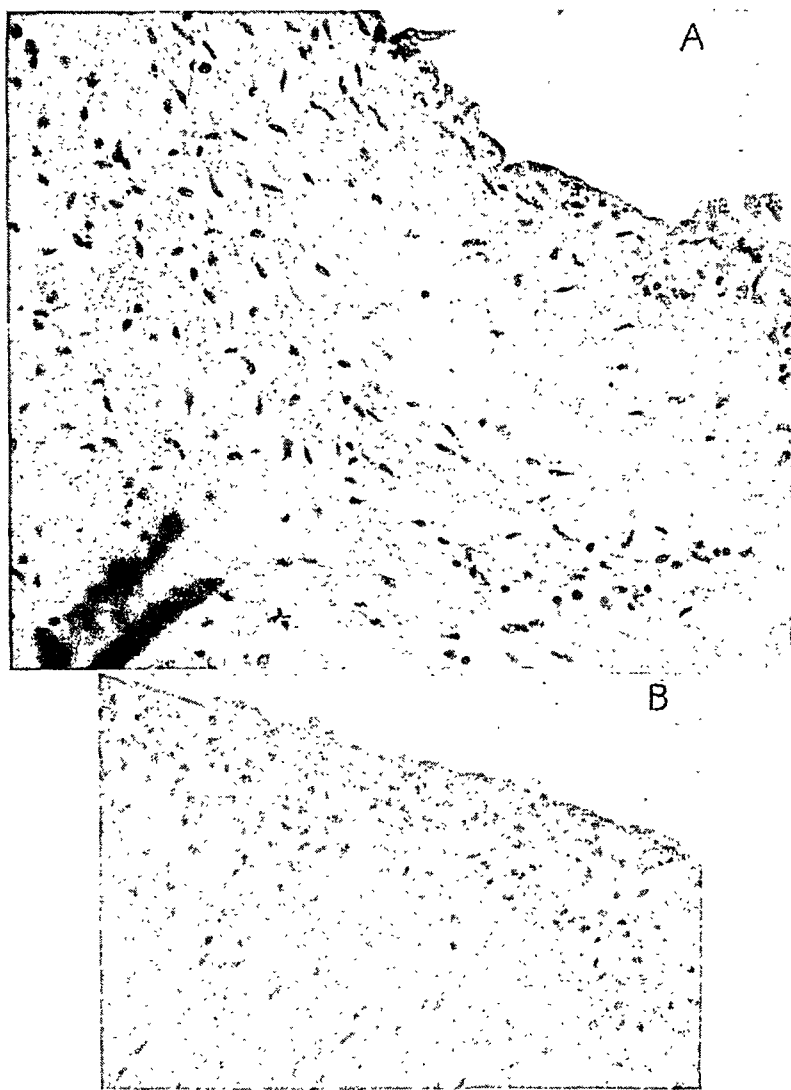


Fig. 6.—*A*, mitral valve in case 35, that of a girl, 5 years old, who died from diphtheria. The valve was macroscopically normal. Note shedding of endothelium, hyalinization and necrosis of endothelial layers, lymphocytic infiltration and subendothelial and deep histiocytic reaction.

*B*, mitral valve in case 40, that of a boy, aged 9, who died from measles complicated by bronchopneumonia. Note marked lymphocytic infiltration and histiocytic reaction in the valvular layers.

and secondary infarction of the lung (17). The lesions were slight in both cases, especially in case 17.

*Group 7.*—In group 7 were included a number of cases of bronchopneumonia (eighteen cases), of which twelve occurred in infants under 2 years of age and six in children above this age.

Of the cases in suckling infants, seven were of fatal bronchopneumonia uncomplicated by other diseases (3, 7, 8, 13, 14, 16 and 21). In some of these cases the valves showed no obvious lesions; in others (13 and 14), there was partial desquamation of endothelium with homogenization of the valvular layers and lymphocytic infiltration, with diffuse, deep-seated histiocytic reaction. In five cases of bronchopneumonia in infants (9, 10, 00, 18 and 21), in which various complications were present (empyema, fibrinous pleurisy, pulmonary infarction with septic softening, pericarditis, meningitis, etc.), the valvular lesions did not appear more serious or widespread than in the first-mentioned cases; here, also, besides some cases in which the valves gave no indication of lesions (9 and 10), there were others (as case 11) in which the valves showed signs of inflammatory reaction.

In six cases of bronchopneumonia in children over 2 years of age (25, 26, 27, 30, 40 and 41) marked lesions were not met with, even in cases with serious complications, in which one would have expected to find particularly severe lesions (27, pneumonia without resolution; 41, sepsis of diplococcal (?) origin; 26, pleurisy and pericarditis). Marked lesions were present only in one case of bronchopneumonia secondary to measles (40); here there was the usual type of lymphocytic infiltration and histiocytic reaction in the valvular layers (fig. 6 B).

*Group 8.*—Three cases (2, 19 and 42) of various forms of tuberculosis in children of different ages comprised group 8. There were scattered lymphocytic infiltrations in the aortic valve in case 42 (ulcero-caseous pulmonary tuberculosis); on the other hand, these were particularly well marked in case 2 (tuberculous mastoiditis, miliary tuberculosis, tuberculous meningitis), in which the aortic valve especially showed evident signs of endothelial desquamation with an intense lymphocytic and histiocytic reaction. The lesions were still more marked and diffuse in case 19 (acinonodular pulmonary tuberculosis with nodular tuberculosis of the liver and spleen), in which both the mitral (fig. 7 A) and the aortic (fig. 7 B) valves were involved in an extensive endothelial desquamation, with intense lymphocytic infiltration and a diffuse histiocytic reaction in the superficial, as well as in the deep, layers of the valve tissue.

In the valvular tissues of infants under 12 months of age dying of diseases of toxic infective type, without demonstrable evidence of sepsis, there were often histologic changes that could not be demonstrated or

suspected macroscopically. These lesions had similar characteristics, and they differed from one another mainly in their distribution and intensity; usually they were confined to a few areas along the valvular margins, so that only an accurate histologic examination revealed them.

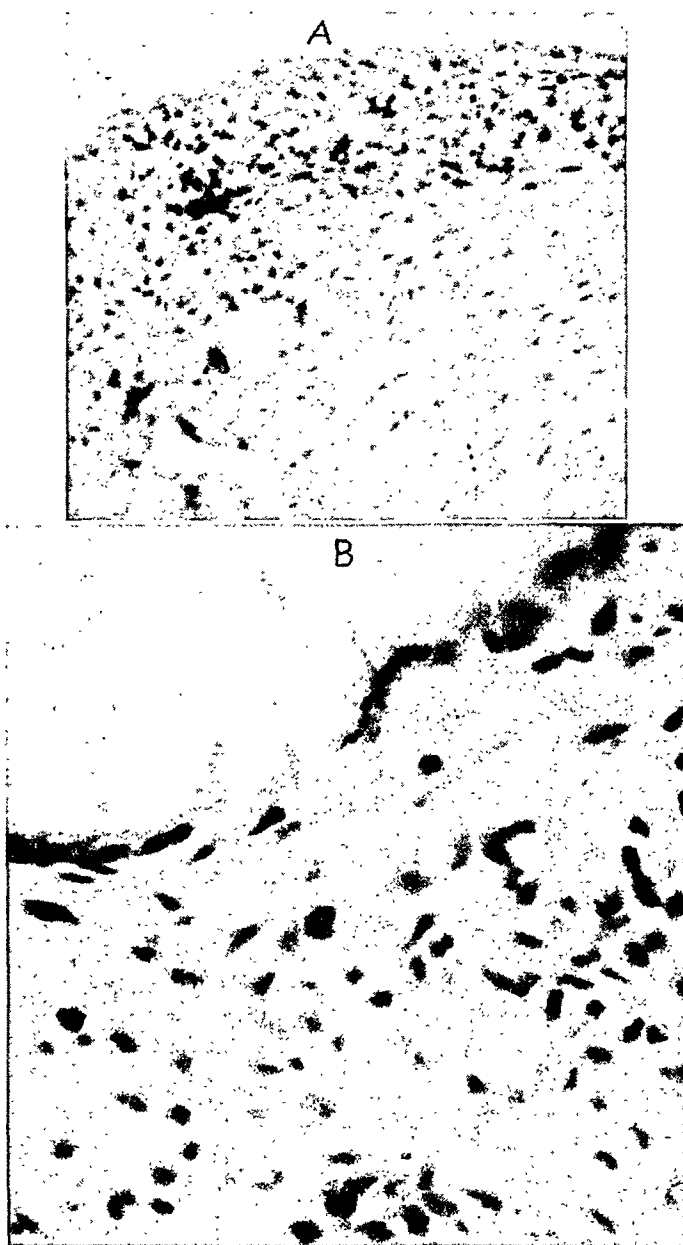


Fig. 7.—*A*, mitral valve in case 19, that of a boy, aged 13 months, who died from acinonodular tuberculosis of the lungs and nodular tuberculosis of the spleen and liver. The photomicrograph shows desquamation of the endothelium, lymphocytic infiltration and deep-seated, diffuse histiocytic reaction.

*B*, aortic valve, showing desquamation of the endothelium, edema, lymphocytic infiltration of the subjacent valvular layers and intense histiocytic reaction.

It often happened that many sections of a valve had to be searched through before a single focus of disease could be met with. Usually the affected areas were situated near the free margins of the valves, where the mechanical effect of the jolting together of the edges of the valves rendered them more susceptible to an inflammatory stimulus; only exceptionally and in the severer cases were the lesions more widely distributed over the valvular tissues.

The lesions consisted in swelling and subsequent desquamation of the endothelium (very rarely, however, it underwent proliferation); edema followed by hyalinization of the underlying connective tissue of the valve, with formation, in some cases, of limited foci of necrosis; lymphocytic infiltration, sometimes in the superficial, but often in the deep, layers, generally on the borderline between the still healthy tissue and the foci of necrobiosis, and finally, active histiocytic reaction corresponding to the lymphocytic infiltration, hence sometimes in the superficial subendothelial layers and sometimes extending also to the deeper layers. Rather exceptionally, there was present a scanty number of polymorphonuclear leukocytes; these occurred only in very severe and relatively advanced cases. Early thrombotic phenomena overlying the valvular lesions were rarely demonstrable.

#### PATHOGENESIS OF LESIONS

The interpretation of the microscopic changes is unequivocal. The changes in the valvular tissues are partly passive (degenerative, necrotic) and partly active (inflammatory). These processes are superimposed and intermingled. One deals with a primary focal valvulitis.

I use the term "valvulitis" instead of "endocarditis" because that term better expresses the nature of the lesions. The word "endocarditis" refers to a more complex process and applies rather to the macroscopic aspect of the lesions produced by the presence of thrombotic vegetations. Hence several authors, following the original researches of Ziegler, insisted rather on the term "thrombo-endocarditis," as if the two factors included in the one term are necessarily connected. So much credence have pathologists given to this that Roesner<sup>7</sup> even considered valvular thrombosis and verrucose endocarditis to be one and the same condition, and more recently Krischner,<sup>8</sup> following the ideas advanced by Beitzke, proposed to classify endocarditis according to the macroscopic appearance and the character of the valvular thrombosis.

This way of regarding endocarditis I have long thought to be objectionable,<sup>3</sup> and the observations here set forth confirm me in this opinion. The formation of the thrombus is a secondary phenomenon which may

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7. Roesner, E.: *Klin. Wchnschr.* **3**:297, 1924.

8. Krischner, H.: *Virchows Arch. f. path. Anat.* **265**:545, 1927.

be entirely absent; hence it cannot be taken as an essential feature of the process. The error of interpretation on the part of authors who have studied the essential nature of the endocarditic process in man has arisen from their observing cases which were apparently in the initial stage, but which actually were far enough advanced to be complicated, and marked, as it were, by the secondary formation of thrombus.

Nor do I believe it correct to accept the views proposed by Jegoroff.<sup>9</sup> He considered septic endocarditis to be the only true form and supposed (but did not prove) this process to be always set up by septic emboli in the blood vessels of the valves, while admitting that these may be in a perfectly normal condition. The milder cases, of the verrucose type, the endocarditis simplex of other authors, which is generally non-microbic (endocarditis toxica), should be considered, according to Jegoroff, not as endocarditis, but as endocardosis, and is caused, not by vascular emboli in the valves, but by toxic agents acting directly on the valvular endocardium.

Leaving aside the serious objections to Jegoroff's pathogenic mechanism of septic endocarditis, I think that the new term "endocardosis" is not acceptable, because it suggests only essentially passive processes in the tissues, whereas one actually has to do in these cases with active processes, characterized by lymphocytic infiltration and histiocytic reaction; that is, processes truly inflammatory. The term "endocarditis," or better, "valvulitis," is therefore to be applied to these conditions because it exactly expresses the nature of the process.

Dietrich,<sup>10</sup> on the other hand, interpreted the process of endocarditis on a reactive inflammatory basis (in the modern sense of the word), and worked out the conception in a series of experiments, following ideas already expressed by Rosenow<sup>11</sup> (namely, that the course and evolution of the infection are due more to a process of bacterial immunization against the antibodies of the host, and so, ultimately, to the original virulence of the germ). These ideas have been adopted by me to explain the pathogenesis of endocarditis,<sup>3</sup> and also by Siegmund<sup>12</sup> in some of his original experiments. Dietrich, producing general infections in animals by vaccination, obtained the phenomena of valvular endocarditis, which he considered to be produced by circulating organisms lodging on existing foci of reaction in the endothelium. The final form of the lesion varies with the modifications

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9. Jegoroff, B.: *Arch. d. mal. du coeur* **19**:647, 1926; *Ztschr. f. klin. Med.* **103**:584, 1926.

10. Dietrich, A.: *Ztschr. f. d. ges. exper. Med.* **50**:85, 1926; *München. med. Wchnschr.* **75**:1328, 1928.

11. Rosenow, E. C.: *Immunological and Experimental Studies on Endocarditis*, *J. Infect. Dis.* **6**:245, 1909; **7**:411 and 429, 1910.

12. Siegmund, H.: *Centralbl. f. allg. Path. u. path. Anat.* **36**:226, 1925.

induced in the organisms by contact with the tissues, that is, with the condition of local immunity in the tissues; hence in the valvular tissues the fate of the process depends on the power of reaction and reabsorption. If these tissues succeed in overpowering the noxious action of the organisms, lesions of the vegetative type result, followed by proliferation of the valvular tissue; but if, on the contrary, the tissues are overpowered, a widespread valvular necrosis occurs, followed by thrombotic deposits and lesions of an ulcerative and polypoid type. Relapses are provoked by alternating changes in the reaction of the tissues. Even endocarditis lenta is not characterized by a strict specificity of organism (*Streptococcus viridans*), but by changes in the mutable endothelial reaction occurring during the course of a chronic and general infection of streptococcic type.

Dietrich's ideas certainly seem acceptable. They confirm and renew the interpretation of endocarditis as inflammatory, putting it in relation with the powers of regulation and defense of the whole organism; and they bring the subject of endocarditis into line with modern views on the pathogenesis of infectious diseases, in general, and of the tuberculous process, in particular.

Some of the points, however, are debatable: First of all, some important experiments by Semsroth and Koch<sup>13</sup> (1929) tended to prove that in the localization of bacteria causing endocarditis, the allergic sensitization preceding the general infection might not be specific, but might be provoked by other causes, such as foreign, nonbacterial protein. Similarly, Klinge<sup>14</sup> obtained sensitization of rabbits toward the rheumatic virus, by using injections of horse serum. Hence, the nodular, degenerative proliferations met with in animals, resembling those found in persons with articular rheumatism (Aschoff bodies), should be considered as a mesenchymal, and not a specific, reaction to the particular organism. These important observations greatly widen one's conception of allergy as a preliminary factor in valvular lesions.

Furthermore, even if the allergic factor has a certain importance in the production of the endocardial lesions, one must not underrate the importance of the elective attraction of a micro-organism and its toxic products toward the valvular tissues. This factor of special attraction is well established by experimental work<sup>15</sup> and occurs in spontaneous disease;<sup>16</sup> there is a tendency for certain types of organism (as compared with others otherwise apparently similar) to give rise to endocarditic lesions.

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13. Semsroth, K., and Koch, R.: Studies on the Pathogenesis of Bacterial Endocarditis, Arch. Path. 8:921, 1929.

14. Klinge, F.: Beitr. z. path. Anat. u. z. allg. Path. 83:185, 1929.

15. de Vecchi, B.: Boll. sc. med. 8:15, 1908.

16. Haden, Russel: J. Lab. & Clin. Med. 12:31, 1926.

Another point may be referred to in regard to Dietrich's conceptions, namely, the presence and import of an endothelial reaction. At least in the initial stages of valvulitis, such as are here described, the endothelium shows no reaction. It only desquamates and disappears, leaving the underlying layers bare. The reaction takes place only in the connective tissue of the valve, with a resultant lymphocytic infiltration and the formation of large elements of epithelioid type, which are to be interpreted as the histiocytes of a reticulo-endothelial reaction, varying in different cases and according to the time the lesions have taken to develop.

A histiocytic reaction in the valvular tissue was noted, though not adequately interpreted, by Koeniger in his classic contribution to the histology of rheumatic endocarditis. This reaction has been confirmed by more recent observations (Darre and Albot<sup>17</sup>), and it has been noticed that in rheumatism and endocarditis lenta an endothelial (or better, "mesenchymal") reaction develops in various parts of the organism (Merklen and Wolff,<sup>18</sup> Istamanowa<sup>19</sup>). This makes the interpretation of the Aschoff bodies in spontaneous and experimental disease clear.<sup>15</sup> Huguenin and Albot<sup>20</sup> demonstrated deep histiocytic reactions in valvular tissues in cases of subacute endocarditis, similar to those met with in the rheumatic forms.

The present observations on the initial stages of acute endocarditis developing in the valves in children as a result of various causal agents confirm these conceptions. The inflammatory reactions show, first, a definite activity of the mesenchyma. Then there is lymphocytic infiltration, and histiocytes appear in the connective tissue of the valves even in areas distant from the superficial layers. The inflammatory reaction in the initial stages of the process is always of the same type, whatever the stimulus. In the later stages, the course and evolution of the process vary according to the nature of the micro-organisms and the resistance of the subject.

#### ETIOLOGY OF THE LESIONS

The importance of the part played by micro-organisms in the development of endocarditis is shown by the fact that in recent years many attempts have been made to arrive at a bacteriologic classification; this conception is not new, but the progress of technic has made it more feasible. For instance, Cowan,<sup>21</sup> in a recent research, classified acute endocarditis as produced by an anhemolytic streptococcus (rheumatism)

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17. Darre, H., and Albot, G.: *Ann. d'anat. path.* **6**:465, 1929.

18. Merklen, P., and Wolff, M.: *Presse méd.* **36**:97, 1928.

19. Istamanowa, T.: *Virchows Arch. f. path. Anat.* **268**:224, 1928.

20. Huguenin, R., and Albot, G.: *Ann. d. path. Anat.* **7**:490, 1930.

21. Cowan, J.: *Glasgow M. J.* **108**:249, 1927.



and by the influenza bacillus. This classification is certainly neither complete nor exact, but would be useful if it could be extended to all observed cases. It would be easier to do this in the clinic than in the autopsy room, where bacteriologic researches are more uncertain and difficult. It is for this reason that, in the present research, save in a few isolated cases, no systematic bacteriologic examinations were made, and so a bacteriologic classification of the cases of incipient endocarditis cannot be attempted. A search for micro-organisms in these sections almost always yielded negative results, and the few positive results do not allow one to draw any useful conclusion. I am obliged to confine myself to a grouping of the cases according to the various types of disease, and this is, of course, an inexact and even fallacious criterion.

In some of the cases observed, especially in groups 1, 2, 4 and 5, it is probable that at a certain period of evolution of the disease, there was a more or less advanced condition of sepsis. In all the definitely grave cases, and in some others in which the valvular lesions were visible macroscopically, the genesis of the lesions is readily explained by the lodging of micro-organisms on the valves owing to some mechanical cause (reciprocal impacts of the valves, formation of whirlpools and eddies in the blood) or to congenital causes (congenital malformations), as has been recently suggested by Grant, Wood and Jones.<sup>22</sup> But acquired morbid changes and antecedent processes cannot be taken as etiologic factors in the case of involvements of the valves in infants and experimental animals.

In the great majority of cases, however, sepsis was not admissible, and the clinical course and anatomic observations do not leave any doubt about this. Even if the micro-organisms were, or had been, present in the circulation, they would have been few, certainly much fewer than in the mild and transitory bacteremia that occurs in almost all acute infectious diseases. It is logical, then, to admit that in all these cases the main factor was the deleterious effect on the valves of the toxic products of the germs (endocarditis toxica), the possibility of the existence of which is admitted by all pathologists, being proved by anatomic, as well as by experimental, observations.<sup>23</sup> With such a genetic hypothesis it is possible to explain the relative mildness of these lesions and the readiness with which they heal.

In the other groups of disease the cases of bronchopneumonia, diphtheria and tuberculosis call for consideration.

*Bronchopneumonia.*—In several cases of bronchopneumonia, both in sucklings and in children, whether uncomplicated or complicated by

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22. Grant, R. T.; Wood, J. E., and Jones, T. D.: Heart **14**:247, 1928.

23. de Vecchi, B.: Arch. anat. pat. **1**:151, 1906. Ravenna, E.: Arch. per le sc. med. **44**:268, 1921.

some other condition, such as pleurisy, pericarditis, meningitis, etc., the valve was healthy to the naked eye, but showed microscopic changes. From these observations I must conclude that the action of the germ which caused the pneumonic process (usually the pneumococcus) was not limited to the pulmonary tissues, but extended to the cardiac valves. It was known that the pneumococcus has the power of acting on the cardiac valves (especially the aortic) and of originating endocarditis, usually vegetative, and of giving rise to grave lesions that usually appear toward the end of the disease or even during convalescence. But it was not known that the initial lesions are so frequently present in the valves during the course of pneumonic processes. It would be useful to ascertain whether similar lesions are also present in adults dying of pneumonia. It is not easy to establish the nature of this lesion; that is, whether it is due to the direct action of the circulating organisms on the valves (there is often a bacteremia in pneumonia), or whether it is a toxic action. The type of the lesions and their extent are in favor of the latter hypothesis.

*Diphtheria.*—As regards the diphtheritic process, the observations bring to light the frequency with which valvular inflammatory lesions occur in the course of this infection. The anatomic and experimental researches on the condition of the heart during diphtheria have been mainly directed toward the investigation of the state of the myocardium, only slight attention being paid to endocardial lesions arising during the course of this disease. The observations of Scagliosi, Fraenkel, Christoph, Howard and others showed that it is possible to find endocarditis of verrucose type in persons who have died of diphtheria. I do not know of really systematic researches on this point. My own experimental investigations<sup>23</sup> on the endocarditis provoked by bacterial toxins proved that the diphtheritic toxin is able to initiate localized inflammatory reactions in the valves in the rabbit, which are followed by the formation of secondary thrombi. These experiments were controlled and confirmed by Ravenna.<sup>23</sup> Systematic observations on the cadaver and some experiments go to prove that the valvular apparatus takes part in the processes induced by the Löffler bacillus, and that the relative rarity of thrombo-endocarditis in persons dying of diphtheria applies only to the most evident and advanced stages of a much commoner and more diffuse process. All the more logical is it to suppose a toxic genesis for this class of changes, as do all authors who wish to attempt an explanation, basing their opinions chiefly on experimental data.

*Tuberculosis.*—Of great interest are the marked changes found in the apparently healthy valves of children affected by tuberculosis.

Experiments (Michaelis and Blum,<sup>24</sup> Bernard and Salomon,<sup>25</sup> my own<sup>26</sup>) have confirmed the old anatomic and clinical observations that the bacillus of Koch can lodge on the valves and originate valvulitis. Michaelis and Blum agreed that it could cause specific histologic lesions (epithelioid and giant cells, foci of caseation)—a claim which should be received with the greatest reserve. But to explain the tuberculous forms of endocarditis that evolve without any possibility of the finding of the bacilli in the thrombus, or with the finding of the bacilli only in very small numbers, another view has been gaining credence—that in the genesis of these valvular lesions in the subjects of tuberculosis it is the toxic action, rather than the direct action, of the germ, in whatever way it comes in contact with the valves. This conception of the toxic origin of tuberculous endocarditis has been confirmed by my experiments, and Mencarelli<sup>27</sup> supported it in recent work in which he especially studied the action of the tuberculosis toxin on the endocardium.

General conclusions may also be drawn in reference to the various kinds of change and their frequency at different ages. From these observations it appears to be clear that in all persons with general toxic infectious diseases, the valves are more frequently and more severely damaged. The cases of endocarditis visible on examination with the naked eye which I have studied were all in children over 2 years of age. This does not apply to the microscopic lesions; very conspicuous and grave lesions have been found even in sucklings (cases 5, 6, 11, 13, 14, 19, etc.), and even in the first few days of life (1 and 2). This shows that at all ages, independent of previous lesions (i. e., the valves being perfectly normal), valvular lesions may develop to a notable intensity and extent, and the evolution of events varies with the circumstances that originate or accompany them.

#### EVOLUTION AND END-RESULTS OF THE LESIONS

All these valvular lesions are to be considered with reference not only to their genesis, but also with reference to the consequences that may follow later. One might suppose that lesions found on microscopic examination occur only in the specially grave cases—that is, in fatal cases. It is more reasonable, however, to think that they occur in all toxic-infective forms of a certain intensity, even in those in which the patient has recovered, and in which the lesions, being relatively superficial and mild, may possibly heal easily and permanently.

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24. Michaelis and Blum: *Deutsche med. Wchnschr.* **24**:550, 1898.

25. Bernard and Salomon: *Compt. rend. Soc. de biol.* **57**:359, 1904.

26. de Vecchi, B.: *Riforma med.* **21**:96, 1921.

27. Mencarelli, L.: *Cuore e circolaz.* **14**:300, 1920.

It is this healing process that is of a special interest, since one cannot imagine a real *restitutio ad integrum* in such delicate tissues as those of the infant's valves, altered and damaged by the destructive and reactive processes, which usually involve the deeper layers of the segments. Possibly superficial valvular lesions, especially those confined to the endothelial and subendothelial layers, might heal quickly and completely, but deeper changes of focal necrosis and lymphocytic and histiocytic infiltration (sometimes very intense) could not disappear without leaving some trace. This is a theory that is readily applied, and Holsti<sup>5</sup> adopted it fully in explaining the pathogenesis of valvular inflammatory changes running a subacute and chronic course.

It is easy to understand, in view of the character of the changes, especially in view of the constant and marked histiocytic reaction, that when the general process has come to an end, healing can take place only by proliferation and substitution of connective tissue, with consequent deformity of the margins of the valves to an extent dependent on the severity of the primary lesions. When the inflammatory process has involved the deeper parts, marked changes must result.

#### CONCLUSIONS

From these researches one may draw the general conclusion that inflammatory processes of marked intensity often take place in young children affected by various toxic infective diseases, but that the subsequent process of thrombosis often fails to appear; thus the macroscopic diagnosis of such lesions at autopsy is almost always impossible. These are lesions of particular importance both for the inferences that can be drawn from them about the pathogenesis of the endocarditic process in general, and on account of the sequelae to which their further development gives rise, in the establishment of valvular defects the mode of origin of which has hitherto been obscure.

# FAT NECROSIS IN BILE PERITONITIS

## EXPERIMENTAL STUDY \*

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During a recent study<sup>1</sup> of the reaction of the peritoneum to sterile bile, fat necrosis was frequently observed in the fat of the mesentery near the pancreas. The term fat necrosis has come to indicate a special form of necrosis of fat tissue which is characterized by a focal circumscribed lesion and by the hydrolysis of the fat in the necrotic area into fatty acids and glycerol, the latter disappearing and the former combining with bases to form soaps. In practically all cases, fat necrosis is produced by the action of pancreatic juice on the adipose tissue. A precise summary of the knowledge of this subject was made by Wells.<sup>2</sup>

Langerhans,<sup>3</sup> in 1890, observed that the fat in the cells is split into its components, and that the fatty acids combine (at least in part) with calcium to form insoluble soaps. Dettmer,<sup>4</sup> in 1895, was able to produce fat necrosis with fresh pancreatic juice, but was not able to do so with a commercial preparation of trypsin. He then concluded that the lipase of the pancreatic juice was the active agent. Flexner,<sup>5</sup> in 1897, supported this contention by demonstrating the presence of the lipase in the foci of fat necrosis. Opie<sup>6</sup> confirmed this observation and was able to demonstrate<sup>7</sup> the presence of a fat-splitting enzyme in the urine of a patient with fat necrosis.

Wells,<sup>8</sup> in 1903, produced typical fat necrosis by injecting extracts of fresh pancreas into animals either of the same species as that from

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1. Rewbridge, A. G.: The Etiological Rôle of Gas Forming Bacilli in Bile Peritonitis. *Surg. Gynec. Obst.* **52**:205, 1931.

2. Wells, H. G.: *Chemical Pathology*. Philadelphia, W. B. Saunders Company, 1925, p. 431.

3. Langerhans, R.: Ueber multiple Fettgewebsnekrose, *Virchows Arch. f. path. Anat.* **122**:252, 1890.

4. Dettmer: Dissertation, Göttingen, 1895.

5. Flexner, S.: On the Occurrence of Fat Splitting Ferments in Peritoneal Fat Necrosis and the Histology of These Lesions, *J. Exper. Med.* **2**:413, 1897.

6. Opie, E. I.: Experimental Disseminated Fat Necrosis, *Johns Hopkins Hosp. Rep.* **9**:859, 1900.

7. Opie, E. I.: A Case of Hemorrhagic Pancreatitis: The Occurrence of a Fat Splitting Ferment in the Urine, *Bull. Johns Hopkins Hosp.* **13**:117, 1902.

8. Wells, H. G.: Experimental Fat Necrosis, *J. M. Research* **9**:70, 1903.

which the pancreas was obtained or of another species. The power of these agents to produce fat necrosis was reduced by heating to 60 C. for five minutes and destroyed at 71 C., indicating that the active agent was an enzyme.

Fat necrosis may be produced by any means that will cause the escape of pancreatic juice from its natural channels. It has followed trauma, acute infections of the gland and the blocking of the ampulla of Vater by gallstones, which permits bile to back up into the pancreatic ducts, where it results in an acute inflammation of the pancreas (Opie<sup>9</sup>).

Flexner,<sup>10</sup> in 1906, showed that it was the bile salts that caused the inflammation when bile passed up the pancreatic ducts, and that this effect was decreased or prevented by large amounts of colloids. As the result of injury by the bile salts, or any other agent that produces death of cells, the injured cells are digested by the pancreatic juice, which then escapes into the surrounding tissue. Wells' experiments showed that the lesions of fat necrosis may be produced in from three to five hours, large enough to be visible to the naked eye; their form and size depend solely on the area of fatty tissue exposed to the action of pancreatic juice.

Schweizer,<sup>11</sup> in 1924, reported a case of bile peritonitis which resulted from a spontaneous perforation of the gallbladder and in which fat necrosis was observed. At necropsy gallstones were present in the gallbladder and cystic duct, but not in the common bile duct or at the papilla of Vater. Both gross and microscopic examination of the pancreas failed to disclose any necrosis or evidence of an inflammatory process. He concluded from this that fat necrosis may occur in the abdomen quite independent of pancreatic disease or of the action of pancreatic juice. The following experiments reproduce the same pathologic changes in dogs that Schweizer observed in man.

#### METHODS

The animals, in the experiments to be described, were electrocuted eighteen hours after their operations. Necropsies were then performed, and the tissues were immediately placed in Zenker's fixative, later embedded in paraffin and stained with hematoxylin and eosin.

#### EXPERIMENT 1

In a series of twenty dogs, after double ligation of the common bile duct and the making of a stroma in the gallbladder, bile was allowed to drain continuously into the peritoneal cavity. Eighteen hours after

9. Opie, E. I.: The Etiology of Acute Hemorrhagic Pancreatitis, *Bull. Johns Hopkins Hosp.* **12**:182, 1901.

10. Flexner, S.: The Constituent of Bile Causing Pancreatitis and the Effect of Colloids on Its Action, *J. Exper. Med.* **8**:167, 1906.

11. Schweizer, R.: Fat Tissue Necrosis with a Perforated Gall Bladder, *Schweiz. med. Wchnschr.* **54**:265, 1924.

the operation the peritoneal cavity of each dog presented the picture of acute, severe general peritonitis and contained several hundred cubic centimeters of a serosanguineous exudate, in which were found polymorphonuclear leukocytes and *B. welchii*. The peritoneal surfaces were inflamed and were covered with a thin layer of fibrin. In the fat of



Fig. 1.—Fat necrosis in bile salt peritonitis.

the mesentery adjacent to the pancreas were areas of fat necrosis about 1 cm. in diameter. Microscopic examination of the pancreas failed to show any evidences of distention of the pancreatic ducts, necrosis of the glandular tissue or any inflammatory exudate in the gland or its stroma. Fat necrosis was observed in fifteen of the twenty dogs in this series.

## EXPERIMENT 2

In a series of twenty dogs the effect of an intraperitoneal injection of a 10 per cent solution of bile salts was then studied. Eighteen hours after the administration of 2.5 cc. of a sterile solution of bile salts per kilogram of body weight there was observed a peritonitis similar

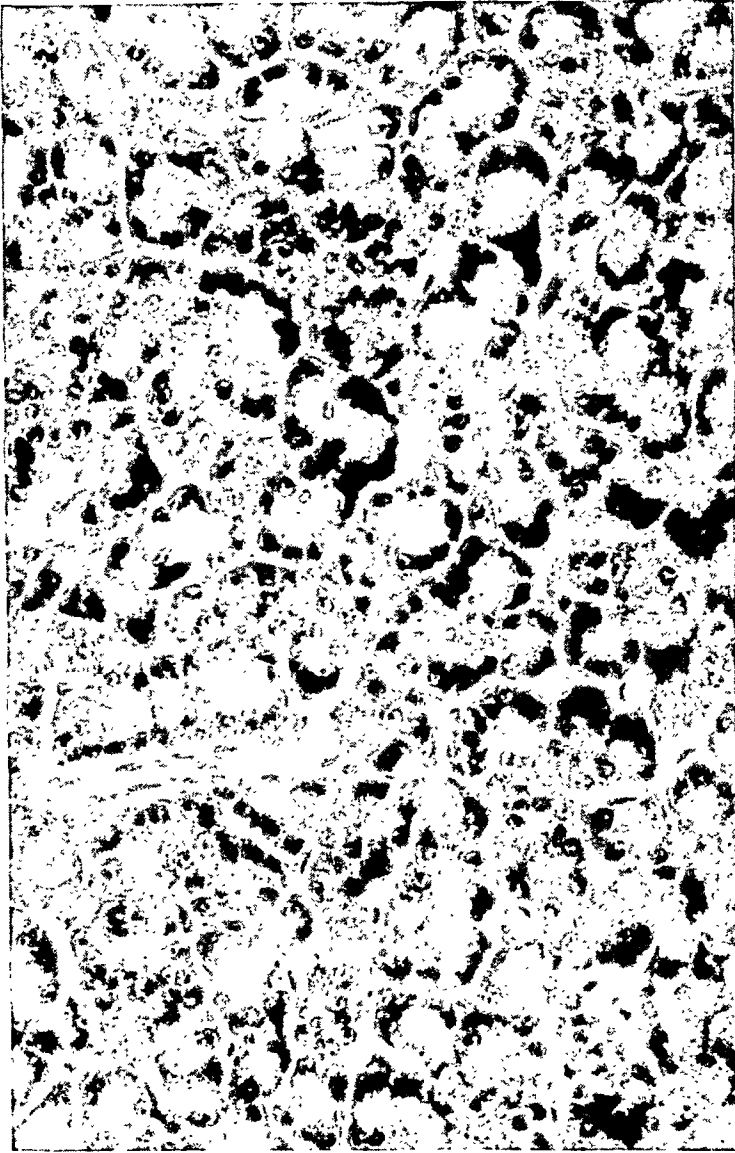


Fig. 2.—Normal pancreas near an area of fat necrosis.

to bile peritonitis, except that the fat necrosis was more extensive, being observed in the fat adjacent to the pancreas, in the mesentery, in the omentum and in the retroperitoneal fat. In this series of twenty dogs fat necrosis was observed in all instances.

The pancreas, when examined grossly, was normal. When examined microscopically no evidence of necrosis of the gland or of distention



of the pancreatic ducts was present. In the adipose tissue surrounding the pancreas were areas of fat necrosis and an occasional accumulation of polymorphonuclear leukocytes, which represented an inflammation secondary to the peritonitis. It is of interest to note, however, that this inflammatory process was confined to the fatty tissue, and that



Fig. 3—Fat necrosis in bile salt peritonitis.

normal pancreatic cells were adjacent to the foci of fat necrosis. The accompanying photomicrographs portray the condition of the pancreases of animals that had extensive areas of fat necrosis produced by the solution of bile salts.

Experiments 1 and 2, in which fat necrosis was observed when bile and a solution of bile salts were introduced into the peritoneal cavity,

tend to show that the bile salts were one of the important factors in its production. However, in the peritoneal exudates produced by bile and bile salts, *B. welchii* was found. In order to determine whether sepsis was involved in its production the following experiment was performed.

#### EXPERIMENT 3

In a series of six dogs peritonitis was produced by the intraperitoneal administration of 20 cc. of an eighteen hour broth culture of *B. welchii*. A peritonitis identical with bile peritonitis was produced, except that fat necrosis was not observed. This experiment tends to show that cultures of *B. welchii*, when introduced intraperitoneally, do not produce fat necrosis.

#### EXPERIMENT 4

The question arose as to whether the bile salts per se were able to produce fat necrosis, or whether the fat necrosis was produced by the local action of the bile salts on the pancreas liberating the pancreatic enzymes and allowing them to diffuse into the surrounding adipose tissue. In a series of six dogs the pancreas was completely excised before introducing intraperitoneally 2.5 cc of a 10 per cent solution of bile salts per kilogram of body weight. The amount of bile salts selected for this experiment had produced fat necrosis in every instance in experiment 2. Although a hemorrhagic peritonitis similar to that of bile peritonitis was produced, no fat necrosis was observed. As a control on this experiment the solution of bile salts was introduced intraperitoneally into three dogs which had had an operation of equal magnitude, such as a partial gastrectomy, and fat necrosis was observed in every instance. This experiment shows that the presence of the pancreas is essential for the production of fat necrosis by the intraperitoneal administration of bile.

#### COMMENT

In Schweizer's case of spontaneous perforation of the gallbladder, the fat necrosis was observed in the omentum. He concluded correctly that fat necrosis may occur independent of pancreatic disease. However, in view of the experiments described in the foregoing paragraphs, there is reason to doubt that it occurs independent of pancreatic juice, as stated by Schweizer. The evidence presented in this investigation shows that bile free in the peritoneal cavity is one of the factors involved in its production. Experiments with solution of bile salts show that the bile salts are the active agents in the bile. *B. welchii* was grown from the exudates produced by bile and bile salts; however, when peritonitis was produced by this organism, no fat necrosis was observed.

When only a few foci of fat necrosis were observed, these foci were always observed in the fatty tissue near the pancreas. When solution

of bile salts was introduced into the peritoneal cavities of depancreatized dogs, no fat necrosis was produced. The pancreases of the dogs with fat necrosis, when examined both grossly and microscopically, were normal. Previous investigations tend to show that fat necrosis is produced by extravasations of pancreatic juice permitting the enzymes to diffuse into the surrounding adipose tissue. Although there is no evidence to show that the bile salts per se are capable of producing fat necrosis, there is evidence to show that the presence of solution of bile salts free in the peritoneal cavity and the pancreas are essential for its production. Since there was no gross or microscopic evidence of necrosis of the pancreas in these experiments, it is necessary to postulate that the pancreatic enzymes were liberated by changes in permeability produced by the local action of the bile salts on the pancreas.

#### CONCLUSIONS

Fat necrosis may be produced experimentally by allowing a sufficient quantity of bile to drain into the peritoneal cavity.

The bile salts are the active agents in the bile.

The presence of the pancreas is essential for the production of fat necrosis by the intraperitoneal administration of bile.

The liberation of the pancreatic enzymes is produced presumably by changes in permeability caused by the local action of the bile salts on the pancreas.

# MYOMA OF THE ESOPHAGUS WITH ASSOCIATED DIVERTICULA

REPORT OF TWO INSTANCES \*

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Leiomyomas occur chiefly in preexisting smooth muscle tissue. In the intestine leiomyomas are not uncommon, but they are rarely seen in the stomach and esophagus.

The association of diverticula with subperitoneal myoma of the intestine was noted by Steiner,<sup>1</sup> but so far as I have been able to determine, such an occurrence has not been described in any of the few instances of esophageal myoma recorded in the literature.

It is true that these tumors are small, relatively rare and often asymptomatic, but the association with diverticula may add to their significance. Benign tumors of the esophagus may assume considerable importance, as in the instance reported by Fahr.<sup>2</sup> In this case the patient died as a result of obstruction to the esophagus by a long, sausage-shaped, pedunculated fibroma which was attached at the origin of the esophagus and extended to the cardia. Three examples of myomas of the esophagus are referred to by Ewing:<sup>3</sup> Pichler's case of multiple leiomyomas with striated muscle cells and ganglion cells, found in the muscular coat; Illig's case of a large myoma of the esophagus in the muscularis; and Eising's case of polypus myoma made up of atypical smooth muscle cells resembling sarcoma, which was also the seat of an early carcinoma. Another example of benign polypoid fibroma was reported by Dyke,<sup>4</sup> in which the patient died as the result of a malignant stricture of the esophagus. At autopsy a soft fibroma, covered with esophageal mucosa, was found attached at the level of the cricoid cartilage. Anitschkow<sup>5</sup> stated that of all fibromas and fibromyomas of the gastro-intestinal tract, the myomas, occurring in the muscular coat of the esophagus near the cardia, are the rarest. These, however, are not clinically significant. He referred to Pichler's case cited here and Ritter's case with malignancy. Anitschkow reported two instances of

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\* Submitted for publication, Jan. 14, 1931.

\* From the Department of Pathology, University of Oregon Medical School.

1. Steiner: Beitr. z. klin. Chir. **22**:1, 1898.

2. Fahr, T.: Klin. Wchnschr. **2**:2347, 1923.

3. Ewing, James: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1927, p. 233.

4. Dyke, S. C.: J. Path. & Bact. **30**:309, 1927.

5. Anitschkow, N. N.: Virchows Arch. f. path. Anat. **205**:443, 1911.

small myomas of the esophagus just above the cardia. He believed that the most probable explanation of their occurrence is on an embryologic basis.

No reference is made to coexistence of diverticula with benign tumors of the esophagus. Jackson and Shallow<sup>6</sup> reported a case of diverticulum associated with a malignant condition of the esophagus.

In view of the fact that no instances of diverticula with related myomas have been recorded in the literature, it seems justifiable to report two cases.

#### REPORT OF CASES

CASE 1.—A woman, aged 32, died at the Multnomah County Hospital of latent corrosive mercuric chloride poisoning. She took poison with suicidal intent and was brought to the hospital in a moribund condition. The mercurial nephrosis had begun to subside when the patient died of diphtheroid and hemorrhagic ileocolitis. The small diverticulum and myofibroma in the lower third of the esophagus were discovered in the course of the routine postmortem examination. There was nothing in the clinical history to indicate that these had ever caused symptoms.

The tumor was 2.5 cm. above the cardia in the left lateral and posterior wall of the esophagus (fig. 1). It was kidney-shaped, with the long axis parallel to the vertical diameter of the esophagus. The concave surface was directed internally toward the lumen of the esophagus and impinged on the orifice of the diverticulum, which it narrowed. The tumor measured 3.5 cm. in length, 1.5 cm. in width and 1.3 cm. in thickness. The diverticulum extended cephalad and was partially surrounded by the tumor, which was arranged over the superior half. This association resulted in a comma-shaped ostium of the diverticulum with the narrow end directed downward. The opening here measured 0.5 cm. by 1.5 cm. The diverticulum was 1 cm. deep and 1.5 cm. wide, and extended 2 cm. upward. The tumor was externally smooth and circumscribed. The cut surface was firm, shiny and whitish.

To study the location and structure of the tumor and diverticulum better the esophageal wall was cut into blocks, each about 2 mm. thick. The blocks, indicated in figure 2 by the letters *A*, *B*, *C*, *D*, *E* and *F*, were embedded in paraffin and sectioned serially. Every tenth section was mounted, stained with van Gieson's connective tissue stain and counterstained with hematoxylin.

Block *A* included the most inferior portion of the tip of the comma-like orifice of the diverticulum. Grossly here the mucosa was eroded at the midpoint, then thickened in the depression where it was folded back near the inferior pole of the tumor. The muscular coats were of relatively uniform thickness. The end of the block near the tumor was thick, owing to the folding of the mucosa and a small accumulation of fat external to the muscular coats.

Microscopic examination of the serial sections revealed definite changes, particularly in the mucosa. The usual stratified squamous epithelium was seen at the narrow end of the section, but extended only about one fourth of the way across the surface to the point of infolding. For a short distance the mucosa was completely absent. Soon, however, there were seen glandlike structures in the scarred, exposed muscularis mucosae. The mucosa here was replaced by an amorphous fibrous and fibrinous substance staining dull blue, and containing many groups of alveoli and crypts. The latter were lined with disintegrating tall columnar cells

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6. Jackson, C., and Shallow, T. A.: *Ann. Surg.* **83**:1, 1926.

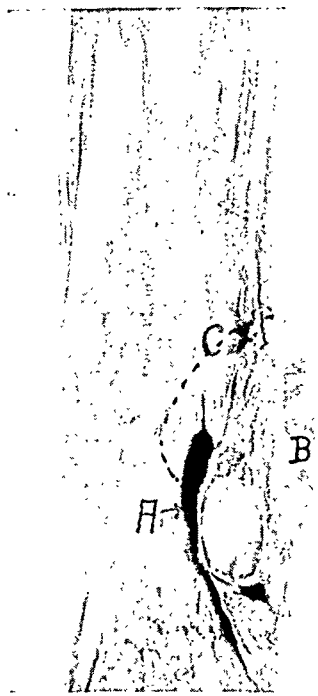


Fig. 1 (case 1).—Esophagus opened along the midline posteriorly: *A* is the orifice of the diverticulum; *B*, the tumor in the lateral and posterior wall. The dotted line *C* indicates the extension of the myoma about the diverticulum.

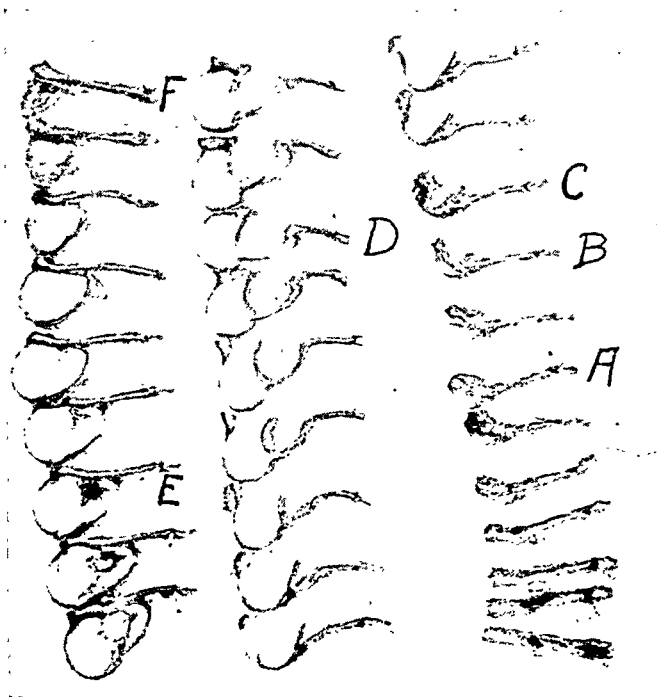


Fig. 2 (case 1).—Serial sections illustrating the relationship of the tumor to the diverticulum. The blocks marked *A*, *B*, *C*, *D*, *E* and *F* were selected for serial microscopic sections.

with small, circular, deeply basic-staining nuclei. The cytoplasm was pale and clear, and occasionally formed goblet cells. The alveoli and crypts increased in number as one approached the infolding at the lower end of the orifice to the diverticulum. Here the entire mucosa was formed by these structures with but little stroma. There was an irregular infiltration of small lymphocytes and a few plasma cells about the alveoli. Such areas closely resembled the structure of the mucous glands of the esophagus. Beyond the infolding the mucosa was similar to that described in the depths of the fold, but the stroma here was abundant and a dull light blue. In the muscularis mucosae at this point there was a localized increase in the smooth muscle. The cells were crowded together and tended to form whorls. The stroma here was relatively abundant. The muscularis elsewhere was unchanged. The muscular coats were of the usual thickness and free from any cellular infiltration.

Section *B* was taken from the wall of the esophagus immediately below the inferior pole of the tumor and prepared and stained as described. The infolding was deeper than in section *A*, and the erosion of the mucosa less pronounced. Microscopically the sections were similar to those of *A*. The glandular structure of the mucosa at the infolding was more pronounced. The mucosa in these sections simulated that of the small bowel. The stratified epithelium at the opposite end of the section covered more of the surface, was thinner, and in one section approached the glandular structures. Here the stratified epithelium was very thin and fragmented, and appeared to extend out over the glandular area. It was separated from the latter by a thin layer of fibrous connective tissue. It was evident that the glandular structure represented an overgrowth of the mucous glands in the muscularis mucosae, the entire mucosa being eroded. There was some distortion of the muscularis beyond the infolding similar to that seen in section *A*. However, the small tumor present here was lost in sectioning. The muscular coat was unchanged.

Gross section *C* was taken through the inferior pole of the tumor. Macroscopically the mucosa was more complete than in the other two blocks. The infolding was deeper, and the modified mucosa of the depression was thicker. The muscular walls appeared thicker about the tumor. The tumor here was located between the muscularis mucosae and the inner circular muscular coat. Microscopically the stratified epithelium was similar to that in section *B*. It covered more of the muscularis and in many sections extended to within a few millimeters of the infolding. The modified mucosa, made up of mucous glands resting on the muscularis, was more abundant and the fold deeper than previously described. Many lymph follicles were seen in the muscularis. The infiltration of the modified mucosa by small round cells was here as in other portions. The tumor at the wide end of the section was situated between the muscularis mucosae and the inner muscular coat. In two of the serial sections the smooth muscle cells of the tumor were seen to be continuous with the inner surface of the inner circular muscle bundles. The tumor was very dense, being made up of closely packed smooth muscle cells and fibrous connective tissue. The smooth muscle cells predominated. These cells were arranged in parallel bundles with an occasional whorl. At this end of the tumor there was an infolding from the inferior surface, leaving a small opening and an incomplete septum partially dividing the main mass. In the small opening, down near the muscular coat, there were many sinuses filled with blood. These formed a small area of hemangiectasis within the base of the tumor. The tumor was loosely encapsulated and was itself relatively avascular. The muscular coat at this point was many times the thickness seen in sections *B* and *A*. There were no other evident microscopic changes.

Block *D* was taken through the upper end of the tumor at the widest portion of the orifice of the diverticulum. Here the tumor was situated in each lateral wall of the diverticulum. The main mass was found at the extreme edge of the section with a small, thin comma-shaped portion in the opposite wall. The mucosa appeared to be thin as it dipped into the diverticulum. The muscular wall was not grossly evident about the walls of the diverticulum.

Microscopically the stratified squamous epithelium was thinned and eroded, but covered the flat surfaces and dipped down into the diverticulum covering almost the entire wall. The floor of the diverticulum, however, was devoid of an epithelial lining. The muscularis was exposed, fibrosed and continuous throughout, but thinned as it dipped down into the walls of the diverticulum. The muscular coat extended to the edge of the small comma-shaped tumor, then became very thin as it extended around the tumor and diverticulum. This small tumor was continuous with the inner layer of smooth muscle and was similar in appearance to previous sections of it. The large tumor mass in the opposite wall was of like structure, having an avascular central portion and a somewhat vascular periphery.

Block *E* was taken through the larger portion of the tumor and the most superior tip of the diverticulum. Here the diverticulum extended to the mucosa, muscularis and muscular coat, as it extended cephalad from the ostium. It was surrounded on three sides by tumor. The mucosa and walls of the esophagus appeared grossly unchanged. The tumor and diverticulum were between the deep layer of the muscular coat and the periesophageal connective tissue. Microscopically the mucosa, muscularis mucosae and muscular coats were unchanged. The serial section of the small, pouchlike tip of the diverticulum revealed it to be lined with stratified squamous epithelium which was slightly thinner than that seen on the surface of the esophagus, but which was otherwise unchanged. The muscularis was seen deep to the ring of stratified squamous epithelium. The tumor was essentially as described previously.

Gross section *F*, the last section of the series, was taken at the most superior tip of the tumor. Grossly no changes were observed in the wall of the esophagus, except for a slight thickening of the mucosa overlying the tips of the tumor. Microscopic sections revealed the presence of several mucous glands beneath the mucosa. These had slender ducts, which passed along obliquely through the muscularis and mucosa. Just proximal to the orifice was an ampulla, which narrowed down to form an orifice of small caliber. Near the thick mucosa over the tumor the ampulla of one duct was dilated, being some five times the size of others. The duct was also dilated, and the gland from which it came was slightly enlarged. Here also the overlying mucosa was thrown into small folds and was scarred. The glands seen in the other portions were located in the loose connective tissue between the muscularis and the muscular coat. The structure of these glands was identical with the modified mucosa occurring in the fold at the orifice of the diverticulum. There were no remarkable changes in the muscular coat.

CASE 2.—A woman, 59 years of age, was found dead at her residence. The coroner's autopsy revealed marked coronary arteriosclerosis, thrombosis of the anterior interventricular branch of the left coronary artery and chronic rheumatic mitral endocarditis with stenosis and insufficiency. During the course of the necropsy a small myoma was discovered in the posterior wall of the lower third of the esophagus. Just above the tumor in the anterior wall there was a shallow diverticulum.

The tumor was located 1.2 cm. above the cardia and was 1.2 cm. in diameter and 1.3 cm. long. It was bean-shaped, with a twisted tapering projection from



right to left in the posterior wall of the esophagus. The diverticulum was 3.5 cm. above the cardia, was oval shaped, and measured 1.5 cm. long, 1 cm. wide, 0.5 cm. deep. The long axis was parallel to the vertical diameter of the esophagus. The esophagus was 4.5 cm. in circumference at the cardia, 3.5 cm. at the tumor and 4 cm. just above the tumor. Gross serial sections were made of this portion of the esophagus and were prepared as in the previous series. The sections selected for

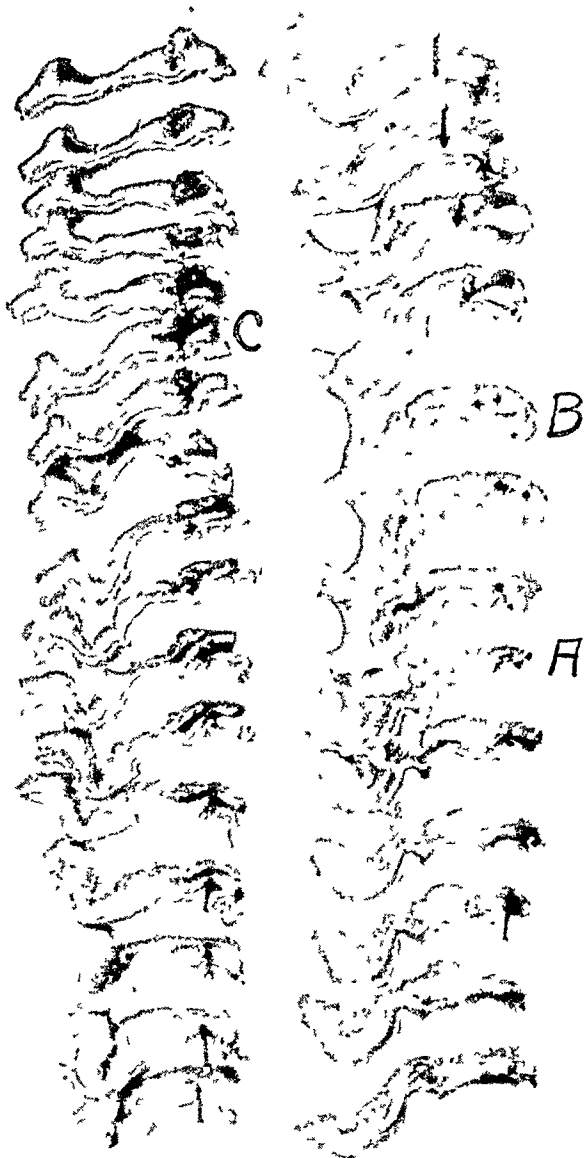


Fig 3 (case 2).—Cross-sections of the esophagus, tumor and shallow diverticulum. The blocks between *B* and *C* show the relationship of the tumor and diverticulum. Note that the association is not as close as that in case 1 (fig. 2).

serial microscopic sections are marked *A*, *B* and *C* in figure 3. Block *A* was from the most inferior pole of the tumor. The mucosa here was wrinkled and intact. The tumor was located in the muscular coat. Microscopic examination of serial sections revealed the mucosa about the tumor to be modified. Here the stratified epithelium was thinned and eroded. For a short distance the stratified squamous

epithelium was absent, and a modified mucosa similar to that described in the fold in case 1 was seen. Likewise there was a large dilated duct, lined with tall columnar epithelium. The muscularis was intact throughout. Between it and the muscular coat were many mucous glands and ducts, as well as an occasional lymph follicle. The tumor at one end of the section was similar in structure to that seen in case 1. It was made up of smooth muscle, a scant stroma and blood supply. At one point the fibers of the tumor were continuous with those of the internal muscular coat. No further changes were noted in the esophageal wall.

Block *B* was taken from the tip of the small twisted end of the tumor. Grossly the mucosa was intact, and the small tumor was indistinctly seen in the muscular coat. Microscopically, the stratified squamous epithelium was intact, except over the tumor, where glandular structures occurred. The mucous glands of the muscularis mucosae were numerous and were slightly infiltrated with small round cells. The mucosa was thrown into folds and at one point formed a small pouch, which was very near the tip of the tumor. Beneath it the tissue was slightly infiltrated with small round cells and was fibrosed. The tumor in this location was small and completely surrounded by the muscular coat. The latter contained in one section another mass similar to the tumor, but much smaller.

Gross section *C* was taken through the shallow diverticulum above the tumor. Macroscopically the mucosa appeared folded, but otherwise negative. The muscular walls were unchanged. Microscopically the stratified squamous epithelium was intact, but was thinned in the region of the diverticulum. The muscularis mucosae was intact and unchanged. The muscular coats revealed no microscopic changes.

#### COMMENT

In both cases the leiomyomas occurred in the inner layer of the smooth muscle and were directly connected with it. The small tumors seen in the second instance were embedded in the smooth muscle, were circumscribed and exhibited pseudo-encapsulation. These tumors were made of orderly whorls of smooth muscle cells very different from the normal wavy smooth muscle about them. Such characteristics, with the finding of other small tumors similar in structure and location to the larger ones, pointed to their congenital origin. Congenital anomalies are known to be multiple rather than singular.

The areas of muscularis covered with mucous glands simulating gastric mucosa described in the sections from both cases probably represented another anomaly. Ewing,<sup>7</sup> in discussing the etiology of carcinoma of the esophagus, referred to islands of mucous glands in the esophageal mucosa of persons showing this condition. Hewlett<sup>8</sup> described these as the superficial glands of the esophagus and considered them to be distinct from the mucous glands beneath the muscularis. He stated that these occur as islands of branched tubular glands lined with columnar epithelium, replacing the stratified squamous epithelium. Microscopically they simulate gastric mucosa, but as the esophageal

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7. Ewing (footnote 3, page 904).

8. Hewlett, A. W.: *J. Exper. Med.* 5:319, 1900-1901.

mucosa is considered by Maximow to develop in situ, they could not be congenitally misplaced gastric mucosa rests. The absence of parietal cells is another point against the gastric origin. Hewlett further stated that such islands of glands occur most commonly at the level of the cricoid cartilage, but the cardiac end of the esophagus may contain them.

The association of leiomyomas diverticula and islands of superficial glands of the esophagus undoubtedly points to a common congenital origin. Diverticula are known <sup>6</sup> to develop at certain ill-supported areas of the esophagus. Others are reported to be of congenital origin. Jackson and Shallow <sup>6</sup> reported such a case and stated that Maylord believed that all congenital diverticula of the esophagus have their origin in persistent second branchial clefts. Steiner <sup>1</sup> found that the associated changes in the lumen of the small intestine depended on the location of the tumor in the wall of the bowel. The submucous type cause stenosis or intussusception, and the subperitoneal produce diverticula. These diverticula associated with the small myomas of the esophagus may be the result of mechanical factors coupled with the congenital defects, evidenced by the tumors and the superficial glands. The enlargement of the tumor and the downward pressure of swallowing may cause a rotation of the tumor with eccentric enlargement and a drawing in of the esophageal mucosa to form the diverticulum. Other factors may be the thinning of the wall about the tumor and the possibility of a congenital defect of the wall as a fourth anomaly.

#### SUMMARY

Two instances of myoma of the lower third of the esophagus with associated diverticula are reported, with their gross and microscopic characteristics, and illustrations are given.

# HEALING OF THE GASTRIC MUCOUS MEMBRANE IN RABBITS AFTER ITS SURGICAL REMOVAL \*

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The present investigation of the healing of experimental lesions of the gastric mucous membrane in rabbits was begun to determine whether there is a tendency in these animals toward the development of chronic gastric ulcer and to consider the mechanical effects of the continuous presence of food in a constantly distended stomach.

Ferguson<sup>1</sup> removed an area of mucosa about 1 cm. in diameter from the middle of the anterior surface of the stomach in rabbits, and allowed them to live for from one to forty days after the operation. There were few signs of regeneration, and an ulcer was found in every rabbit of the experiment. He suggested that the rabbit's stomach is never empty, that the underlying suture in the muscular and serous coats acts as a foreign body, and that there is an intrinsic lack of regenerative ability in the gastric epithelium.

Continuing the work begun by Ferguson, Fauley and Ivy<sup>2</sup> removed an area from the posterior, and one from the anterior, wall of the stomach in rabbits, through an incision in the anterior wall. In thirty days the simple lesion of the posterior wall had healed, but there was a chronic ulcer on the anterior wall at the site of the silk suture. These rabbits were fed normal rough food. In spite of the silk suture, however, only three of seventeen rabbits which were fed soft food had ulcers thirty days after the removal of areas of mucosa from the anterior walls of their stomachs.

Nicolaysen<sup>3</sup> froze an area in the anterior gastric wall in rabbits with ethyl chloride for from three to eight minutes. This injured all layers, not merely the mucosa. Most of the animals that lived, some for six months, had typical chronic peptic ulcers.

## METHOD OF EXPERIMENTS

In operating on the rabbits, aseptic technic and ether anesthesia were used. The animals were allowed a diet of alfalfa hay, oats and water, with no restriction of the quantity eaten. Two types of operation were performed: removal of an area

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\* Submitted for publication, Dec. 9, 1930.

1. Ferguson, A. N.: *Am. J. Anat.* **42**:403, 1928.

2. Fauley, G. B., and Ivy, A. C.: *Proc. Soc. Exper. Biol. & Med.* **27**:531, 1930.

3. Nicolaysen, Knud: *Deutsche Ztschr. f. Chir.* **167**:145, 1921.

of mucous membrane from the region of the fundus and removal of an area of mucous membrane from the region of the pylorus.

(*Removal of Area from the Fundus.*—The abdomen was opened in the median line, from the xiphoid process to the umbilicus. Two mosquito forceps were attached to the anterior wall of the stomach, one near the greater curvature at about its middle, the other 3 cm. from the first, toward the lesser curvature. The stomach was lifted up into the incision with these forceps, and the remainder of the abdominal cavity was carefully protected by moist gauze packs. The serous and muscular coats were incised between the points at which the forceps were attached. By retracting the combined serous and muscular coats, the underlying mucous membrane was readily exposed over a large area by blunt dissection. Then, with scissors, a circular piece of mucous membrane was removed, and the opening into the stomach was immediately packed with gauze to stop hemorrhage and to prevent leakage of gastric contents. Usually a piece of gastric mucosa about 2.5 cm. in diameter was removed; however, the size could not be determined exactly because the mucosa always seemed to stretch as it was being exposed. The incision in the wall of the stomach was closed by one layer of continuous suture through the combined serous and muscular coats. In some of the experiments silk sutures were used; in others, chromic catgut (no. 00), and in still others, plain catgut (no. 00). The omentum was always laid over the suture line in the stomach. The incision in the abdominal wall was closed with three layers of continuous silk suture.

(*Removal of Area from the Pylorus.*—The abdominal incision was the same as in the first procedure. Two mosquito forceps were attached to the anterior surface of the pyloric portion of the stomach, one at the upper edge of the thickened band of pyloric muscle: the other, 2 cm. from the first, toward the fundus. With a small scalpel, an incision 2 cm. long was made between the two forceps through all layers of the gastric wall, and the mucous membrane on the lesser curvature at the upper edge of the pylorus was grasped in the forceps. By lifting gently on the forceps, the mucous membrane was separated from the underlying muscle, and a round area was snipped off with scissors. Areas from 0.5 to 1.2 cm. in diameter were removed. The incision in the gastric wall was sutured with plain catgut (no. 00) to make a transverse closure; the mucosa was closed with a continuous suture, and the muscle and serosa were inverted with a mattress suture. The incision in the abdominal wall was closed as in the first procedure.)

## RESULTS

Twenty animals used in the first series of experiments lived longer than 20 days, and were dispatched at intervals of from 21 to 121 days after operation. One animal died of pneumonia and empyema 100 days after operation, but as the mucosa was completely healed, the experiment is included in the consideration of results. Practically all of the rabbits that were allowed to live for a long time gained weight and seemed healthy.

The gastric mucous membrane of nine of the twenty-one rabbits was found to be completely healed. Twelve rabbits had chronic gastric ulcer at the site of operation (table 1). All of these lesions, however, showed definite evidences of healing. The ulcers were much smaller than the original pieces of mucous membrane removed. None was

greater than 1 cm. in diameter; some were relatively deep and had perforated the muscular coat. The omentum was usually adherent to the serosa at the site of the lesion. The margins were slightly elevated, and overhung the base of the ulcer. On microscopic examination of histologic sections, regenerating epithelium was seen at the edges of the ulcers, as reported by Caylor<sup>4</sup> in his description of the healing ulcer of human beings. The general picture of these gastric ulcers in rabbits is similar to that described by Mann<sup>5</sup> for healing gastrojejunal ulcers in dogs.

The shortest time in which complete healing occurred was forty-nine days. For purposes of comparison, the animals that were killed

TABLE 1.—*Results After Excision of an Area of Mucous Membrane from the Fundus of the Stomach*

Rabbit	Time Allowed to Live After Operation, Days	Dimensions of Ulcer, Cm.*	Type of Suture in Wall of Stomach
1.....	21	0.8 by 0.8 by 1.0	Silk
2.....	27	0.6 by 0.6 by 0.5	Silk
3.....	28	0.3 by 0.3 by 0.2	Plain catgut
4.....	28	0.8 by 0.5 by 0.3	Plain catgut
5.....	33	0.3 by 0.3	Silk
6.....	49	None	Silk
7.....	50	None	Plain catgut
8.....	57	0.4 by 0.4 by 0.4	Silk
9.....	57	0.4 by 0.3 by 0.2	Chromic catgut
10.....	64	None	Silk
11.....	64	None	Plain catgut
12.....	71	None	Chromic catgut
13.....	72	None	Silk
14.....	74	0.8 by 0.8 by 1.0	Silk
15.....	85	0.2 by 0.1 by 0.1	Plain catgut
16.....	91	1.0 by 0.8	Silk
17.....	99	None	Chromic catgut
18.....	100	None	Silk
19.....	101	0.3 by 0.3	Silk
20.....	106	0.2 by 0.1 by 0.1	Chromic catgut
21.....	121	None	Silk

\* The area of gastric mucosa removed was approximately 2.5 cm. in diameter.

after forty-nine days were considered separately in groups according to the type of suture material used in closing the wall of the stomach. In each group, healed lesions and ulcers were about equal in number to those in the other groups, indicating that the type of suture used in the wall of the stomach apparently had no relation to the failure of the mucosa to heal (table 2). Also, although in some instances a piece of silk suture was hanging in the lumen of the stomach from the base of the ulcer, in others, pieces of silk suture extended through healed mucous membrane. It is certain that some of the ulcers were not caused by the presence of the suture as a persistent mechanical irritant,

4. Caylor, H. D.: Ann. Surg. **86**:905, 1927.

5. Mann, F. C.: S. Clin. North America **5**:753, 1925.

and that some of the areas healed in spite of the retained suture material.

Of the rabbits used in the second\* experiment, nine were killed at intervals of from 25 to 114 days after operation. In two rabbits, killed, 25 and 43 days after operation, respectively, ulcers were found at the sites of the removal of mucosa. In all the other animals of the series, the mucous membrane of the lesser curvature of the stomach was healed; in four of them, however, a chronic ulcer was present at the site of the gastrotomy (table 3). In all the operations of this type, the incision in the stomach was closed with sutures of plain catgut (no. 00).

TABLE 2.—*Relation of Type of Suture in Wall of Stomach to Healing*

Days After Operation	Silk		Chronic Catgut		Plain Catgut		Total	
	Healed	Ulcer Present	Healed	Ulcer Present	Healed	Ulcer Present	Healed	Ulcer Present
0 to 48	0	3	0	0	0	2	0	5
49 to 121	5	4	2	2	2	1	9	7
Total	5	7	2	2	2	3	9	12

TABLE 3.—*Results After Excision of an Area of Mucous Membrane from the Pyloric Region of the Stomach*

Rabbit	Time Allowed to Live After Operation, Days	Dimensions of Area of Mucosa Removed, Cm. (Approximately)	Dimensions of Ulcer	
			At Site of Removal of Area of Mucosa, Cm.	At Site of Gastrotomy, Cm.
22	25	0.5 by 0.5	1.0 by 0.2 by 0.1	None
23	43	1.2 by 0.7	1.0 by 0.8 by 0.2	None
24	50	1.0 by 0.8	None	1.2 by 0.7 by 0.2
25	84	0.5 by 0.5	None	1.0 by 0.7 by 0.1
26	88	1.0 by 0.5	None	None
27	103	0.8 by 0.8	None	1.5 by 0.5 by 0.2
28	105	1.0 by 1.0	None	None
29	114	1.0 by 0.8	None	0.1 by 0.1 by 0.1
30	114	0.8 by 0.8	None	None

Therefore, failure of the mucosa to heal completely was not due to the presence of nonabsorbable suture material in the underlying tissues. In one animal the lesion of the lesser curvature was entirely healed, and the mucosa had healed at the point of gastrotomy to form a tight sheath around several stiff hairs which were projecting into the stomach.

#### COMMENT

After removal of an area of mucosa from either the region of the fundus or that of the pylorus of the stomach of a rabbit, healing occurs much more slowly than in dogs or cats. Experiments involving the latter two animals have been reported by Morton,<sup>6</sup> Carnot,<sup>7</sup>

6. Morton, C. B.: *Ann. Surg.* **85**:207, 1927.

7. Carnot, Paul: *Compt. rend. Soc. de biol.* **94**:637, 1926.

Ferguson, Dragstedt,<sup>8</sup> Dragstedt and Vaughn<sup>9</sup> and Wilensky and Geist.<sup>10</sup> This was indicated by Ferguson, whose studies did not extend over so long a time after operation as the experiments now being reported.

As the areas of mucosa removed from the region of the (pylorus) were smaller than those removed from the region of the (fundus,) the rates of healing could not be exactly compared. Healing occurred more quickly, however, in the area in the lesser curvature of the stomach, in the region of the pylorus. Morton showed, in dogs, that this region is most susceptible to the development of chronic ulcer. Either this rule does not hold true in regard to rabbits, or some other factor was responsible for the development of chronic ulcers in the present experiments.)

The presence of the suture line directly underneath the lesion of the gastric mucosa undoubtedly was significant in delaying healing. This was suggested in the series of operations on the fundus, but was more forcibly indicated in the operations in which an area was removed from the lesser curvature, near the pylorus. Chronic ulcers developed in almost half of the latter cases, under the scar of the incision, even though mucous membrane was not removed from that site. The presence of hair and other roughage in the gastric content and the presence of nonabsorbable suture material in the wall of the stomach did not seem to be significant factors in delaying healing. Probably the increased connective tissue at the site of the incision, resulting in impaired circulation, was the chief cause of the development of ulcers.

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8. Dragstedt, L. R.: Contributions to the Physiology of the Stomach: XXXVIII. Gastric Juice in Duodenal and Gastric Ulcers, *J. A. M. A.* **68**:330, 1917.

9. Dragstedt, L. R., and Vaughn, A. M.: Gastric Ulcer Studies, *Arch. Surg.* **8**:791, 1924.

10. Wilensky, A. O., and Geist, S. H.: Experimental Studies in the Production of Chronic Gastric Ulcer, *J. A. M. A.* **66**:1382, 1916.

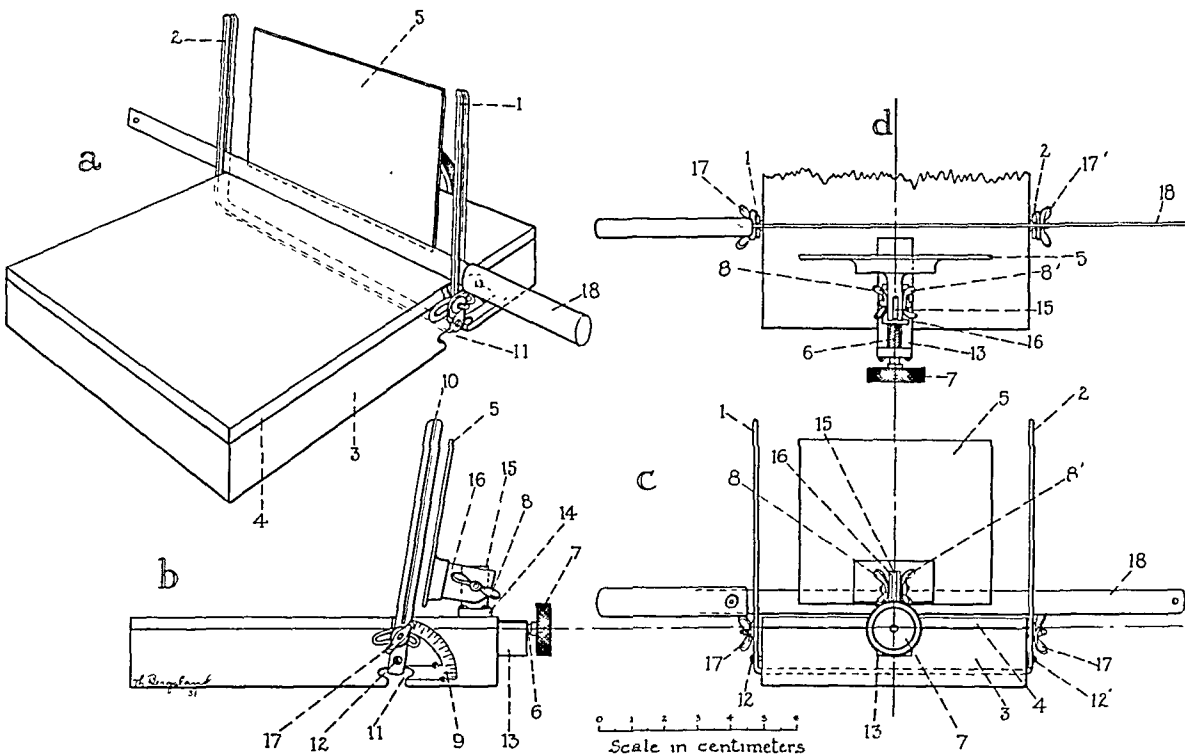


# Clinical and Occasional Notes

## A NEW BRAIN SLICER\*

H. C. STEVENS, M.D., CLEVELAND

The device which is here described makes possible the accurate cutting of uniformly thick gross sections of the hardened human brain, varying in thickness from 5 mm. to several centimeters. By orienting the brain with reference to the plate, 5 (see figure, *a*), sections may be cut in the horizontal, vertical (sagittal) and transverse (coronal) planes. Owing to the fact that the longitudinal axis of the brain is a curve with



Working drawing of brain slicer, one-seventh actual size.

the concavity downward, it is desirable to be able to cut transverse sections at right angles to this curve. This may be achieved by angulating the knife with successive sections through the brain from the anterior to the posterior portion. The plate, 5 (see figure), is essential for the cutting of thin sections, because it forms a firm, flat surface against which the brain is pressed. In order that the plate may be used when the knife is angulated, it is evident that the plate also must be capable of rotation, so that its surface may be kept parallel with that of the knife.

\* Submitted for publication, April 9, 1931.

\* From the Institute of Pathology, Western Reserve University.

The apparatus which is shown in different views in the figure (*a, b, c, d*) consists, in essentials, of two slotted knife-guides, 1 and 2, which are mounted on an oak base, 3, covered with linoleum, *n*. A brass plate, 5, can be advanced or withdrawn by means of the screw, 6, which is turned by knurled head, 7. Angulation of the brass plate, 5, is brought about and maintained by means of two winged nuts, 8 and 8'. The degree of angulation of the knife-guides is measured on the protractor, 9, and fixed by the winged nuts, 17 and 17'. The plate is made parallel to the knife by sighting through the slots, 10, of the knife-guides. The slotted knife-guides are made from one piece of strap iron, 5 by 12 mm. in cross-section, which is bent in the form of a broad letter U. The horizontal part of this piece of metal is placed in a slot, 11, cut out of the under surface of the oak base, 3. The axis about which the knife-guides rotate is that of the screws, 12 and 12'. The dimensions of the base are 27.5 by 20 by 5 cm. The covering of linoleum is 6 mm. thick. The knife-guides are 20 cm. long. The slot in the knife-guides is 2 mm. wide. The screw, 6, permits a travel of 6.3 cm. This screw, which is 1 cm. in diameter, is housed in an oblong brass box, 13, 8 by 3.5 cm., made of a brass plate 0.5 cm. thick. A brass block, 14, which travels on a screw, 6, carries a brass tongue, 15, which is placed in a narrow cleft of 16, and gripped tightly by nuts 8 and 8'. The plate, 5, is made of brass 15 by 12.7 by 0.2 cm. The fiber of the wood is filled with melted paraffin to prevent absorption of liquids. The knife, 18, which is 34 by 2.5 by 0.1 cm., was made from a piece of hack-saw blade of Sheffield steel.

Another very simple device to aid in slicing the brain was described by Anderson.<sup>1</sup>

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1. Anderson, J.: *How to Stain the Nervous System*, Edinburgh, E. & S. Livingstone, 1929.

# Historical Review

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## CARDIOVASCULAR RENAL DISEASE

REPORT OF A CASE OF THREE THOUSAND YEARS AGO \*

ALLEN R. LONG, M.D.

BUFFALO

Although a good deal of work has been done on the gross anatomy and histology of mummies, the nature of the material has not usually been such that the diagnosis of a disease affecting several organs has been possible. The case here reported seems to be an example of the condition commonly called cardiovascular renal disease, occurring in an elderly person 3,000 years ago, who also had marked pneumonokoniosis, with encapsulated caseous areas in one lung.

Through the courtesy of Mr. H. E. Winlock of the Metropolitan Museum of Art, New York, the organs of seven Egyptian mummies were received in this laboratory. They were excavated from a tomb used for women connected with the royal family during the Twenty-First Dynasty (about 1,000 B.C.), at Deir el Bahri in ancient Thebes.

The mummy in question was that of a person named Teye, whose age, according to Mr. Winlock, seemed to be about 50 years. The hair was white, the skin of the abdomen wrinkled, and the bones were much rarefied, but the manubrium was not joined to the sternum.

Eight organs were submitted for examination. Of the eight it was possible to identify beyond question both lungs, the intestines, the kidneys, the heart and the aorta.

The heart was the most satisfactory. It needed to be handled with great care. It was about the size of a hen's egg, not much thicker than an egg shell and much more fragile. The mitral valve was located and photographed; the chordae tendineae and papillary muscles were plainly shown (fig. 1). There was a small calcified mass on one leaflet indicating old endocarditis.

The coronary arteries showed well marked fibrous thickening, chiefly of the intima, with good-sized patches of calcification (fig. 2). The striae on the cardiac muscle fibers were photographed. There were areas of fibrous tissue in the cardiac muscle, like little scars. It

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\* Submitted for publication, Jan. 13, 1931.

\* From the Department of Pathology, University of Buffalo School of Medicine.

is probable that the woman suffered from cardiac pain that would be called angina pectoris today.

The aorta showed moderate nodular arteriosclerosis. Sections of the aorta took specific stains for elastic tissue well.



Fig. 1.—The heart, showing the mitral valve; natural size.

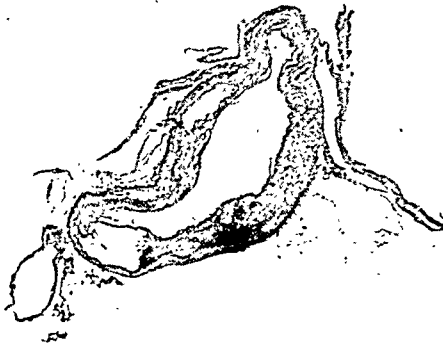


Fig. 2.—A coronary artery: a low power photomicrograph;  $\times 16$ .

Both lungs presented marked pneumokoniosis. Bits of cartilage from the bronchi were demonstrable. One lung had a few areas of caseation that were well encapsulated by fibrous tissue, but no tubercle bacilli were demonstrable.

The microscopic structure of the intestinal wall was recognizable only as far as its layers were concerned. The contents were miscellaneous vegetable fibers of no particular interest. No parasites were found.

Both kidneys were recognized by their histologic structure; many glomeruli were fibrous. There was a thick capsule and a large amount of interstitial fibrous tissue with arteriosclerosis of medium-sized arteries. No nuclear stain was secured in any of the sections.

In the package that should have contained liver were found two large pieces of tissue that appeared to have been varnished. Neither of them appeared like the livers from the other mummies. Each of these pieces contained a large mass of adipocere. In the examination of a large number of sections, no clue to the identity of the organ could be discovered. Only a quantity of adipose tissue could be recognized. The suspicion may be entertained that the ancient embalmers lost the liver belonging to the lady Teye, and that a mass of putrid tissue from some animal was substituted. It is also suggested that she may have been more than 50 years of age.

# General Review

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## BACTERIAL METABOLISM, C

### A SUMMARY\*

ARTHUR ISAAC KENDALL

CHICAGO

Bacteria, the smallest and simplest in structure of known independent living things, are very close to that shadowy hiatus between the quick and the dead. In them the phenomena of life are reduced to the most primitive terms. They have no sex, no morphologically distinct nucleus,<sup>1</sup> and all the essential functions of growth, multiplication and purpose in the scheme of life are carried on by single cells, the average diameter of which is rather less than 1 micron (0.001 mm.), and of which a single one would weigh about 0.000,000,002 mg.<sup>2</sup>

In the study of the microscopic forms known as bacteria, we have what might fitly be called the focal point of the various branches of biological science. Though their investigation may require careful morphological researches, yet the unmistakable monotony of form, combined with a considerable variation of physiological activity, has compelled the bacteriologist to pay much attention to means by which such physiological variations may be more or less accurately registered in order that they may serve as a supplementary basis for classification. Again, with the unicellular organisms, the manifestations of cell activity become the most important phenomena for study. These manifestations bring together the fields of Physiology and Chemistry, and make Bacteriology in one sense a branch of Physiological Chemistry.<sup>3</sup>

Thus wrote Theobald Smith thirty-eight years ago. At that time, physiologic chemistry was a promising department of physiology, and bacteriology was making its spectacular appearance in the world of science as a foster child of pathology. Now, after four decades, the

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\* Submitted for publication, Jan. 21, 1931.

\* From the Department of Research Bacteriology, Northwestern University Medical School.

\* Studies in Bacterial Metabolism have appeared as follows: I-VII, *J. Biol. Chem.* **12**:13, 19, 215, 219, 465, 469, 1912; *ibid.* **13**:63, 1912; VIII, *Med. Rec.* **84**:151, 1913; IX, *Boston M. & S. J.* **168**:825, 1913; X, *J. Infect. Dis.* **13**:425, 1913; XI, *J. Biol. Chem.* **15**:277, 1913; XIII, *J. M. Research*, n. s. **23**:465, 1913; XIII-XXX, *J. Am. Chem. Soc.* **35**:1201, 1913; XXXI-XXXVIII, *ibid.* **36**:1937, 1914; XXXIX-XCIX, *J. Infect. Dis.* **15-47**: (incl.), 1914-1930.

1. The bacterial cell is rich chemically in nuclear substance, however.

2. Kendall, A. I.: *Civilization and the Microbe*, Houghton Mifflin Company, 1923, p. 17.

3. Smith, Theobald: *The Fermentation Tube*, Wilder Quarter Century Book, 1893.

import of these prophetic asseverations has become more generally comprehended. The amazing developments in physiologic chemistry and in bacteriology have opened vistas which lead forward to the understanding and the conquest of disease, and to the harnessing of microbic activity to the projects of man. They reach backward to a primitive pattern of life itself. The chemical changes induced by various kinds of bacteria in their environment during growth and multiplication afford important information of the chemical pattern of life in general, and in particular as it relates to animals and to man.

#### GENERAL PHENOMENA OF BACTERIAL METABOLISM

The metabolism of any living organism consists of two distinct phases: a structural phase in which the organism reproduces and replaces its traditional and hereditary architecture, and an energy phase during which the organism characteristically performs certain actions consonant with its place on the ladder of life. In the bacteria these two phases, while somewhat overlapping, are readily distinguishable chemically, with respect both to the character of the products formed and to the nature of the processes involved. The structural phase precedes the energetic phase in point of time, but this interval may be very brief indeed. For example, with the aid of the microscope, cholera vibrios, growing under optimal conditions of temperature and nutrition, may actually be seen to divide into two mature organisms every fifteen minutes. This rate of multiplication, if continued under favorable conditions, would mean ninety-six generations in twenty-four hours, with a theoretical daily progeny of nearly  $8 \times 10^{28}$ , an immense number: thus a person may be exposed to cholera and die of it within eighteen hours. Fortunately, many natural barriers—exhaustion of food, accumulation of waste products, mutual antagonism—restrain microbic growth to compatible limits.

The phenomena of this microbic growth are important. Phylogenically, the cholera organism creates an exact replica of itself every fifteen minutes over a period of time. This involves among other substances, the fabrication of that exquisitely complex and specific hereditary protein architecture in which resides the élan and the individuality of the organism. Chemically, this proteinogenesis is a series of hydrogenic condensations in which simpler amino-acids and peptides are elaborated into complex specific proteins through the union of the smaller molecules, usually with elimination of hydrogen and oxygen in proportion to form water.<sup>4</sup> The actual amount of nitrogenous material required

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4. Kendall, A. I.: Bacteriology, ed. 3, Philadelphia, Lea & Febiger, 1928, p. 68.

to produce a complete microbe is extremely small, as the following calculation will show:

*Bacillus coli* is an average-sized organism. It measures approximately 1 micron in diameter and 2 microns in length. Its specific gravity is about 1.030. Its weight, has been shown previously,<sup>5</sup> is approximately 0.000,000,002 mg. Five hundred millions of colon bacilli, therefore, would weigh scarcely 1 mg. About 85 per cent of the total weight of a bacterium is water, hence the substance not water, of 500,000,000 colon bacilli is but about 0.15 mg., an extremely small amount. It follows then that even with a very generous allowance for waste, the quantities of materials required to furnish the structural needs of half a billion fully mature colon bacilli are expressible in small fractions of a gram.

In disproportionate contrast to the minuteness of the average-sized bacterial cell is its capacity to induce extensive chemical changes in its environment. The physiologic basis for this phenomenon lies partly, but not entirely, in the ratio of the surface area of the bacterium to its weight or mass.

The structural needs of living things are, in general, directly related to their mass; their energy requirements, on the other hand, are a function of the ratio of their surface area to their mass. A comparison of these ratios for *B. coli* and for man will be illuminating:

$$\frac{\text{B. coli surface}}{\text{B. coli weight}} : \frac{0.000,01 \text{ sq. mg.}}{0.000,000,002 \text{ mg.}} \quad \frac{\text{man surface}}{\text{man weight}} : \frac{1.5 \text{ sq. M.}}{70 \text{ kilograms}}$$

In the microbe, this ratio of 5,000 to 1 contrasts sharply with the corresponding ratio or relative equality for man. A word of caution must be interjected at this point. The ratio of surface area to weight, while very important, does not explain all the phenomena associated with microbic mass and concomitant microbic chemical change. For example, *B. dysenteriae*, almost exactly the same size as *B. proteus*, induces far less chemical conversion per microbe-milligram hour and per microbe-molecule disruption in mediums from which carbohydrate is excluded, than the latter. This is in keeping with an observation of Theobald Smith<sup>6</sup> that, in general, pathogenic bacteria are chemically less reactive than saprophytic bacteria. In corresponding mediums to which utilizable carbohydrate has been added, these organisms are mutually transformed into producers of lactic acid. Under these conditions their metabolic activities, both qualitatively and quantitatively, approach equality more closely.<sup>7</sup>

5. Footnote 4, p. 26.

6. Smith, Theobald: Am. Med. 8:711, 1904.

7. Kendall, A. I.: Am. J. M. Sc. 156:157, 1918.



## QUALITATIVE PHENOMENA OF BACTERIAL METABOLISM

From the earliest days of bacteriology, it has been known and recognized that bacteria, each after its kind, "ferment" certain sugars. They fail to "ferment" other sugars. The precision with which these fermentative reactions may be reproduced indicates that a very fundamental phenomenon is involved. Herein lies both a method for the recognition of bacteria through their consistent utilization of certain sugars, and conversely, a method for the recognition of certain sugars through their consistent utilization by certain bacteria.<sup>8</sup> *B. alcaligenes* and a few other microbes do not utilize any known carbohydrates for energy. They are those exceptions in the bacterial family which afford additional evidence of the correctness of the phenomena just mentioned. Another important concept flows from these fermentative reactions of bacteria. Most bacteria produce noteworthy amounts of acidic substances

TABLE 1.—*Products of Action of Various Bacteria on Mediums (1) Containing and (2) Not Containing, Utilizable Sugar*

Organism	Products When Growing in Medium in	
	Presence of Utilizable Sugar	Absence of Utilizable Sugar
<i>B. alcaligenes</i> *	Ammonia; endotoxin	Ammonia; endotoxin
<i>B. coli</i>	Lactic acid	Indol, phenols, ammonia
<i>B. proteus</i>	Lactic acid	Indol, ammonia, soluble proteolytic enzyme
<i>B. welchii</i>	Lactic and butyric acids	Soluble, specific toxin
<i>B. diphtheriae</i>	Lactic acid	Soluble, specific toxin

\* *B. alcaligenes* cannot utilize (ferment) any sugar.

(conspicuous among which is lactic acid) from the utilization of these sugars, whereby they are automatically reduced to a nearly uniform type of chemical activity. Even so, however, their biologic specificity is of course maintained. In absence of utilizable carbohydrate, but under otherwise parallel conditions, each microbe produces from the common nitrogenous constituents of cultural mediums chemical substances which are specific for each kind of organism; otherwise they would be mutually chemically indistinguishable. Stated differently, it appears that the chemical individualities of at least a majority of the commonly known bacteria depend on their utilization of nitrogenous substances for both energy and structural requirements.<sup>7</sup> This is shown qualitatively in table 1. Two highly important facts stand out clearly. In presence of both the usual nitrogenous constituents of cultural mediums and, in addition, a utilizable carbohydrate, the several bacteria cited, with the significant exception of *B. alcaligenes* (which it will be remembered ferments no sugars), produce lactic acid in common. A

8. Kendall and Yoshida: J. Infect. Dis. 32:355 and 362, 1923.

chemical analysis of these cultural mediums freed from bacteria would therefore afford little or no information of the identity of the microbe in each case, as putrefactive products or substances indicative of the utilization of proteins for energy are entirely absent. This absence of putrefactive products in cultures of bacteria grown in carbohydrate mediums has long been recognized through the observations of Péré,<sup>9</sup> Kruse,<sup>10</sup> Smith,<sup>11</sup> Marshall<sup>12</sup> and many others.

In absence of carbohydrate, but under otherwise parallel conditions, each organism, after its kind, generates distinctive products of growth. *B. coli*, for example, forms indol, phenolic bodies and ammonia; *B. proteus*, indol, ammonia and a powerful, soluble proteolytic enzyme; *B. welchii* and *B. diphtheriae* each a potent, specific soluble toxin. A chemical analysis of these carbohydrate-free mediums, after removal of bacteria, would therefore reveal the identity of the microbe involved. This Dr. Jekyll-Mr. Hyde phenomenon is so generally encountered among bacteria parasitic on or pathogenic for man it may be stated unequivocally that these bacteria, while utilizing carbohydrate for energy, generate in common lactic acid as their characteristic product of metabolism. These same bacteria, utilizing protein derivatives in place of carbohydrate for their energy, produce nitrogenous substances that are chemically specific. The phenomena mentioned refer to conditions under which there is respectively either an excess or an absence of utilizable carbohydrate. If a small quantity of carbohydrate is present in a culture medium, an amount which can be completely fermented by a specified microbe before accumulation of acid restrains its further growth, the initial product of metabolism is, of course, lactic acid. When the sugar is exhausted, the bacteria of necessity turn to the protein ingredients of the medium for their energy requirements. The products of metabolism then include, in addition to lactic acid, gradually increasing amounts of specific, nitrogenous products of metabolism, the chemical composition of which depends on the organism under consideration. Some practical applications of this Dr. Jekyll-Mr. Hyde phenomenon will be discussed later.

#### QUANTITATIVE PHENOMENA OF BACTERIAL METABOLISM

There is quantitative chemical proof, as well as qualitative chemical evidence, of the correctness of the doctrine that bacteria, as here indicated, utilize carbohydrate in preference to nitrogenous substances

9. Péré: Ann. de Inst. Pasteur 6:512, 1892.

10. Kruse: Ztschr. f. Hyg. u. Infektionskr. 17:1, 1894.

11. Smith: J. Exper. Med. 2:543, 1897.

12. Marshall: J. Hyg. 7:581, 1907.

for energy, when both are simultaneously available. The chemical analyses recorded in table 2 show the quantitative changes induced in the nitrogenous constituents of cultural mediums by four representative bacteria cultivated both in presence and in absence of dextrose. The methods of analysis<sup>13</sup> and the details of each organism<sup>14</sup> are published elsewhere. The results are expressed in milligrams of nitrogen per hundred cubic centimeters of culture medium.

Table 2 shows comparatively and in detail the nature and the extent of the changes induced by *B. dysenteriae*, Shiga, *B. typhosus* and *B. coli*

TABLE 2.—Quantitative Changes Induced in Nitrogenous Constituents of Plain Medium and Medium Containing Dextrose by Given Bacteria

		Results of Analyses, Mg. per 100 Cc. of Medium								
Organ- ism	Nitrogenous Constituents of Medium	Con- trol	Plain Medium				Dextrose Medium			
			1 Day	3 Days	6 Days	10 Days	1 Day	3 Days	6 Days	10 Days
B. dysen- teriae, Shiga	Total nitrogen.....	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
	Protein nitrogen.....	716	716	758	780	757	716	768	842	790
	Nonprotein nitrogen..	284	284	243	221	243	284	231	158	210
	Polypeptid nitrogen...	200	213	167	139	158	194	157	74	127
	Aminonitrogen.....	32	32	27	32	32	27	25	38	38
	Ammonia nitrogen....	53	52	48	50	52	54	51	46	46
B. typho- sus	Total nitrogen.....	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
	Protein nitrogen.....	715	743	754	854	808	733	743	850	827
	Nonprotein nitrogen..	285	261	249	146	197	270	261	155	176
	Polypeptid nitrogen...	198	163	155	41	91	182	167	62	95
	Aminonitrogen.....	39	45	41	40	36	41	47	48	37
	Ammonia nitrogen....	47	53	54	65	72	45	45	45	45
B. coli	Total nitrogen.....	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
	Protein nitrogen.....	716	726	790	810	789	726	790	830	800
	Nonprotein nitrogen..	284	273	210	189	210	273	211	168	199
	Polypeptid nitrogen...	200	190	129	87	106	191	135	87	117
	Aminonitrogen.....	32	21	11	26	26	30	23	35	37
	Ammonia nitrogen....	54	63	69	73	79	53	47	47	47

in the nitrogenous constituents of the cultural mediums, both in presence and in absence of dextrose, and after one, three, six and ten days' incubation at 37 C. These changes are in general progressive up to and including the sixth day, when the cultures have reached their maximal growth. By the tenth day, recessive changes and autolysis have taken place. For brevity, therefore, discussion is limited chiefly to the analyses for the six days' growth.

*Metabolism of B. Dysenteriae, Shiga, B. Typhosus and B. Coli.*—In Presence of Dextrose: The outstanding and significant feature of the growth of the dysentery, typhoid and colon bacilli, respectively, in the dextrose-containing mediums is an increase in the protein nitrogen frac-

13. Kendall, A. I.: J. Infect. Dis. **30**:211, 1922.

14. On *B. dysenteriae* Shiga, see Kendall and Haner: J. Infect. Dis. **30**:225, 1922; on *B. typhosus*, *ibid.*, p. 232; on *B. coli*, Kendall and Bly: *ibid.*, p. 239.

tion and a concomitant, almost molecule-for-molecule decrease in the polypeptid nitrogen fraction. This is readily seen in table 3. In the dextrose culture of the dysentery bacillus, the protein nitrogen fraction increased 126 mg. in six days, and the polypeptid fraction was concomitantly reduced 126 mg.; in the culture of *B. typhosus*, the gain in protein nitrogen was 135 mg. and the loss in polypeptid nitrogen was 136 mg.; the gain in the protein nitrogen fraction for *B. coli* was 114 mg., corresponding to a loss of 113 mg. for the polypeptid nitrogen fraction. The aminonitrogen and the ammonia nitrogen fractions of the mediums containing dextrose remained practically constant during this six day period of growth. The total nitrogen content also remained unchanged. The explanation for this shifting of polypeptid nitrogen fraction to protein nitrogen fraction, therefore, is very simple and direct.

TABLE 3.—*Bacterial Changes Induced in Nitrogenous Constituents of a Medium Containing Dextrose*

Organism	Nitrogenous Constituent	Results of Analyses, Mg. per 100 Cc. of Medium		
		Control	After 6 Days	Gain or Loss
<i>B. dysenteriae</i> , Shiga	Protein nitrogen....	716	842	+126
	Polypeptid nitrogen	200	74	—126
<i>B. typhosus</i> .....	Protein nitrogen....	715	850	+135
	Polypeptid nitrogen	198	62	—136
<i>B. coli</i> .....	Protein nitrogen....	716	830	+114
	Polypeptid nitrogen	200	87	—113

These organisms, utilizing dextrose for their energy requirements, have multiplied rapidly. Nitrogen in some form is indispensable for the structural needs of bacteria. The bacteria have selected polypeptid nitrogen and woven it molecule for molecule into the complex protein nitrogen of their bodies. For this purpose neither the aminonitrogen nor the ammonia nitrogen fractions of these cultural mediums are as suitable as the ingredients which comprise the polypeptid nitrogen. The aminonitrogen and the ammonia nitrogen fractions therefore remain quantitatively unchanged.<sup>15</sup>

**In Absence of Dextrose:** In absence of dextrose, when the bacteria must obtain both their energy and structural requirements from the

15. Miss Stephenson (Bacterial Metabolism, London, Longmans, Green & Company, 1930, p. 218) makes the surprising assertion that "ammonia is undoubtedly the chief nitrogenous product of the bacterial decomposition of protein, but it is also the chief nitrogenous food of bacteria: . . . the decrease of ammonia in bacterial cultures on broth, meat, etc., to which carbohydrate has been added, is to be attributed in the main to the increased bacterial multiplication due to an additional and readily available source of carbon."

nitrogenous constituents of the mediums, the course of events is somewhat different, as is clearly indicated in table 4.

Here again, there is a very material increase in the protein nitrogen fractions, indicative of the increase in the bacterial population after six days' growth. With the exception of *B. dysenteriae*, this increase in protein nitrogen is more than compensated for by losses in the polypeptid fraction. In other words, the polypeptid loss exceeds the gain in protein nitrogen. The excess loss in the nonprotein fraction is found chiefly in the increase of the ammonia fraction of the cultural mediums. This is deamination. It is a measure of the amounts of nitrogenous constituents of the mediums used by the bacteria for their energy requirements. Ammonia as such has no energy value for microbic growth.<sup>16</sup> It is eliminated from the peptid molecules prior to their oxidation for the energy needs of the microbes, and it consequently collects as ammonia in the cultures. This is shown very clearly by the

TABLE 4.—*Changes Induced in Nitrogenous Constituents of Plain Medium by Given Bacteria*

Organism	Gain or Loss on Six Days' Incubation, Mg. per 100 Cc. of Medium			
	Protein Nitrogen	Polypeptid Nitrogen	Amino-nitrogen	Ammonia nitrogen
<i>B. dysenteriae</i> , Shiga.....	+ 64	— 61	0	— 3
<i>B. typhosus</i> .....	+139	—157	+ 1	+ 18
<i>B. coli</i> .....	+ 94	—113	— 6	+ 19

analyses. It may be remarked parenthetically here that there is no accumulation of ammonia in those cultures in which dextrose is available as a source of energy. Returning to *B. dysenteriae*: it will be noted that the protein nitrogen increase in the culture of this organism is associated with an almost identically equivalent loss in polypeptid nitrogen. In this respect, the dysentery bacillus appears to differ chemically in its metabolism from *B. typhosus* and *B. coli*. It will be recalled, however, that *B. dysenteriae* (Shiga variety), unlike most other parasitic and pathogenic bacteria, can utilize the carbohydrate moiety of the protein molecule<sup>17</sup> for its energy requirements. Hence the dysentery bacillus is using carbohydrate for energy, even in dextrose-free mediums, and its metabolism here should, and does, approach that of a culture of this microbe in a medium containing dextrose.

Comment on Metabolism of *B. dysenteriae*, Shiga, *B. typhosus* and *B. coli*: These studies of metabolism in sugar-free mediums and in mediums containing dextrose reveal one important common feature:

16. Except for certain types of soil bacteria. Cf. Waksman: *J. Bact.* **7**:231, 1922.

17. Kendall and Farmer: *J. Biol. Chem.* **12**:13 and 215, 1912.

in each instance the polypeptid fraction of the medium is by far the most readily utilized, both for the structural and (in absence of dextrose) for the energy needs of the organisms herein described. The totality of discernible nitrogenous change in the mediums containing sugar is a molecule-for-molecule loss of polypeptid nitrogen for a corresponding gain in protein nitrogen. In the sugar-free mediums, with the exception of *B. dysenteriae*, for reasons previously mentioned, the polypeptid fraction is used not only for the structural needs of the newly formed bacteria as indicated by the gain in protein nitrogen, but also for their energy needs. This is shown by the reciprocal loss of polypeptid nitrogen and the concomitant gain by deamination (ammonia nitrogen). These observations are in harmony with the well attested fact that a great majority of bacteria intimately associated with man and the animals grow far better in mediums containing peptones, which are relatively rich in polypeptid nitrogen, than they do in mediums containing either single amino-acids (and therefore rich in aminonitrogen) or in mediums that have ammonium salts as sources of nitrogen. The latter are, of course, rich in ammonia nitrogen.

*Metabolism of B. Proteus.*—*B. Proteus* differs from the bacteria just described in that it is a much more vigorously growing microbe<sup>18</sup> and also in that it produces, in absence of utilizable carbohydrate, a very active soluble proteolytic enzyme. This enzyme brings about deep-seated changes in the nitrogenous constituents of cultural mediums; hence for relative completeness, the metabolism of *B. proteus* is considered here. The experiments with *B. proteus* were of two kinds (1) cultivation of *B. proteus* in mediums containing graded amounts of dextrose, from 0 to 1.5 per cent, and (2) the addition of sterile filtrates of these cultures to sterile gelatin in the proportion of 1 part culture-filtrate freed from bacteria to 19 parts sterile gelatin. Three days' incubation of these mixtures of culture filtrates and gelatin was allowed, to permit maximum change before the chemical analyses were made. One half of table 5 contains the details of the distribution of nitrogen in the cultures at the start and after twenty-four hours', three, five and seven days' incubation at 37 C.

Table 6 taken from the first half of table 5 shows the changes expressed in milligrams per hundred cubic centimeters, which *B. proteus* induces in the total nitrogen fraction of the several mediums, in presence and in absence of varying amounts of dextrose. The analyses are those of the first, third, fifth and seventh days of incubation.

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18. For a description of the *B. proteus* group, see Kendall, Cheetham and Hamilton: J. Infect. Dis. 30:251, 1922.

Table 6 shows clearly that the protein nitrogen fraction diminishes steadily and rapidly, although the retarding effect of 0.75 per cent dextrose is noticeable, both in time and in amount. This loss in the

TABLE 5.—*Nitrogenous Metabolism of B. Proteus and of Its Soluble, Proteolytic Enzyme*

Results of Analyses, Mg. per 100 Cc.													
Nitrogenous Constituent	Day	B. Proteus, Culture						B. Proteus, Soluble Enzyme					
		Dextrose						Dextrose					
		Control	Plain	0.1 %	0.5 %	0.75 %	1.5 %	Control	Plain	0.1 %	0.5 %	0.75 %	1.5 %
Total.....	1	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein.....		721	531	564	676	721	743	803	482	420	639	703	795
Nonprotein.....		280	470	437	325	280	258	195	514	588	369	300	205
Polypeptid.....		209	389	361	253	213	191	151	485	540	325	258	161
Aminonitrogen....		39	32	28	32	29	30	30	37	36	35	35	32
Ammonia.....		32	49	48	40	38	37	12	12	12	10	10	12
Total.....	3	1,000	1,000	1,000	1,000	1,000		1,000	1,000	1,000	1,000	1,000	
Protein.....		721	239	239	453	531		803	348	168	436	520	
Nonprotein.....		280	762	762	548	470		195	648	840	571	488	
Polypeptid.....		209	647	647	469	400		151	568	747	516	441	
Aminonitrogen....		39	36	36	30	28		30	66	78	45	44	
Ammonia.....		32	89	89	49	42		12	13	15	12	12	
Total.....	5	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein.....		721	161	147	239	340	822	796	244	247	410	500	788
Nonprotein.....		280	840	847	762	661	179	202	752	752	587	499	211
Polypeptid.....		209	663	664	648	567	103	158	687	682	526	433	168
Aminonitrogen....		39	33	31	35	30	39	30	50	52	49	50	30
Ammonia.....		32	144	149	79	64	37	13	15	14	14	14	13
Total.....	7	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein.....		721	38	94	94	105	732	796	240	293	393	393	740
Nonprotein.....		280	963	907	907	896	269	202	752	706	604	604	260
Polypeptid.....		209	723	687	730	722	185	150	688	624	530	531	210
Aminonitrogen....		39	36	25	40	33	44	36	55	60	56	55	33
Ammonia.....		33	204	195	137	141	39	19	16	20	19	19	19

TABLE 6.—*Changes Which B. Proteus Induces in Total Nitrogen Fraction of Plain Medium and Mediums Containing Dextrose*

Results of Analyses, Mg. per 100 Cc.						
Days	Control	Plain	0.1 Dextrose	0.5 Dextrose	0.75 Dextrose	1.5 Dextrose
1	721	531	564	676	721	743
3	721	239	239	453	531	...
5	721	161	147	239	340	822
7	721	38	94	94	105	732

protein nitrogen is caused by the activity of the proteolytic enzyme, and as it occurs in spite of the fact that bacteria are multiplying rapidly, it is an indication of the potency of the enzyme. The rapid decrease of protein nitrogen shown in the second half of table 5, where the enzyme acting alone is studied, is another means of estimating its potency. Coincident with the decrease in protein nitrogen, there is a corresponding, but greater, loss in the polypeptid fraction (first half of table 5), with the exception of the medium having 1.5 per cent dextrose. Here the protein fraction increases from 22 mg.

the first day of incubation, to a maximum of 101 mg. the fifth day, when the culture seems to be at its greatest luxuriance. The polypeptid nitrogen of the 1.5 per cent dextrose culture decreases with the gain in protein nitrogen and almost exactly molecule for molecule. This is in accord with the observations made on *B. dysenteriae*, *B. typhosus* and *B. coli* in mediums containing dextrose. It is an expression, not only of the building of new bacterial bodies, but also (when considered in connection with the corresponding figures for the mediums having less dextrose) of the fact that 1.5 per cent dextrose is a greater amount than this strain of *B. proteus* can utilize before the accumulation of acids from fermentation arrest its growth. Stated differently, *B. proteus* can, and does elaborate a soluble, proteolytic enzyme in mediums containing up to 0.75 per cent dextrose. The greater the amount of dextrose up to this amount, the slower is the appearance of the enzyme.

TABLE 7.—Changes in Ammonia Nitrogen Induced by *B. Proteus* in Plain Medium and in Mediums Containing Dextrose

Days	Results of Analyses for Ammonia Nitrogen, Mg.					
	Control	Plain	0.1 Dextrose	0.5 Dextrose	0.75 Dextrose	1.5 Dextrose
1	32	49	48	40	38	37
3	32	89	89	49	42	..
5	32	144	149	79	64	37
7	33	204	195	137	141	39

Turning to the other nitrogenous changes: it is apparent first of all that the aminonitrogen fraction varies but little during the first seven days of growth; that is, during the period when the cultures are most vigorous. In fact, the maximum change noted, a decrease of from 39 to 25 mg. (seven day culture in 0.1 per cent dextrose), is registered in presence of a decrease in protein nitrogen from 721 mg. to 94 mg.—in other words, a loss of 627 mg. This disproportion between the loss in protein nitrogen and the concomitant insignificant change in aminonitrogen seems to explain the negative results obtained by Berman and Rettger<sup>19</sup> and others who have relied on the formaldehyde (Sørensen) titration method in their study of the effects of utilizable sugars on bacterial metabolism.<sup>20</sup>

Aside from the rapid degradation of the protein nitrogen, the most striking alteration in the nitrogenous constituents is the increase in ammonia nitrogen. The respective amounts for the first week of incubation are as shown in table 7.

19. Berman and Rettger: J. Bact. **3**:389, 1918.

20. Cf. also Jones: J. Infect. Dis. **27**:169, 1920.



The increase of ammonia nitrogen in the plain and 0.1 dextrose mediums is rapid, and at the end of seven days is very great indeed. The effects of increasing amounts of dextrose are very clearly shown. Even 0.75 per cent of this sugar, in presence of considerable fermentation acid produced by its utilization, failed to restrain the action of the bacilli on the nitrogenous constituents when the dextrose was used up. On the other hand, in presence of sufficient dextrose (1.5 per cent) deamination did not occur.

It would appear from this, that other observers, who state that formation of ammonia (deamination) does not occur in presence of dextrose because of the accumulation of acid, are in error: the accumulation of acid, is, of course, an indication that the bacteria are utilizing the sugar for energy; otherwise there would be no acid. The continuance of the formation of acid is measured by the residuum of sugar. If the amount of sugar is sufficient to supply the microbes until

TABLE 8.—*Nitrogenous Metabolism of the Soluble Enzyme of B. Proteus in Plain Medium and in Medium Containing Dextrose*

Nitrogenous Constituent	Gain or Loss on Five Days' Incubation, Mg. per 100 Cc. of Medium				
	Plain	0.1 Dextrose	0.5 Dextrose	0.75 Dextrose	1.5 Dextrose
Protein nitrogen.....	—552	—547	—386	—296	— 8
Polypeptid nitrogen.....	+529	+522	+368	+275	+10
Aminonitrogen.....	+ 20	+ 22	+ 19	+ 20	0
Ammonia nitrogen.....	+ 2	+ 1	+ 1	+ 1	0

they have formed enough acid from its fermentation to inhibit their further activity, the protein ingredients of the medium are not changed to any great extent, other than to build up the bodies of bacteria as hitherto stated. If, however, the sugar is exhausted before sufficient acid is generated from it to inhibit further activity, the nitrogenous ingredients are then of necessity utilized for energy, and products indicative of this utilization of protein accumulate. This is shown very clearly in the first half of table 5.

The second half of table 5 contains the analytical data concerning the action of the soluble bacteria-free enzyme of *B. proteus* on sterile (carbolic) gelatin. The several samples were taken at the time when the various mediums were analyzed.

The two most striking facts brought out by the analytical figures for the actions of the enzyme are (1) the reduction of the protein nitrogen fraction, in all except the medium having 1.5 per cent of dextrose, with an almost exactly concomitant increase, in each instance, of polypeptid nitrogen, and (2) the absolutely fixed ammonia nitrogen, which does not in any instance vary more than 2 mg. from the controls. Table 8, showing the losses in protein nitrogen, the gains in polypeptid

nitrogen and the consistent values for aminonitrogen and especially for ammonia nitrogen, will make this statement clear. The amounts are recorded for the fifth day of incubation only: at this time the enzymatic action was apparently maximal.

It will be seen that the action of the soluble, proteolytic enzyme of *B. proteus* is not restrained even by 0.75 per cent dextrose, since the loss of protein nitrogen in the experiment resulted in a total decrease of 296 mg. of protein nitrogen from a total of 796 mg., or about 37 per cent. At the same time, however, this amount of dextrose (0.75 per cent) retarded and restricted the formation of enzyme markedly. This is shown in the corresponding loss in the medium having but 0.1 per cent dextrose, in which the protein nitrogen decreased from 796 mg. to 244 mg., a loss of 552 mg., or 68 per cent. The medium having 1.5 per cent dextrose showed a loss of but 8 mg. of protein nitrogen from a total of 796 mg., which is about the limit of precision of the method.

Turning to changes in aminonitrogen: it is noted that the amount found in mixtures of plain medium and enzyme and of enzyme and medium containing 0.1 per cent dextrose shows an increase of about 20 mg., in contrast with a loss in protein nitrogen, of some 550 mg. This is reduced to zero in the mixture of enzyme and broth containing 1.5 per cent dextrose. It appears that this soluble enzyme of *B. proteus*, in reducing the protein constituents of the medium, has a deep-seated action, reminiscent in this respect of trypsin rather than of pepsin.<sup>21</sup>

Most significant of all the data relating to *B. proteus* are those for ammonia nitrogen. Five day cultures of *B. proteus*, except in medium containing 1.5 per cent dextrose, showed increments of ammonia ranging from about 100 mg. in the medium having 0.50 and 0.75 per cent dextrose, to nearly 200 mg. in the medium having either no dextrose or 0.1 per cent (first half of table 5). This increase in ammonia is due to the deamination which precedes the utilization of the nitrogenous constituents of the medium for energy.<sup>22</sup> It will be recalled that there was an increase of but 6 mg. in the corresponding medium having 1.5 per cent dextrose. The sterile filtrates of these several cultures, which, with the exception of the one of the culture in medium containing 1.5 per cent dextrose, have a very strongly proteolytic enzyme, do not in any instance increase the ammonia nitrogen more than 2 mg. (second half of table 5). The striking difference between the action

21. For a comparison of the enzyme of *B. proteus* with pepsin and trypsin, see Kendall and Keith: *J. Infect. Dis.* **38**:193, 1926.

22. Cf. also Sears: Thesis, Stanford University, January, 1916.

of the living organisms plus enzyme in culture and the enzyme without living organisms in the mixture of sterile gelatin and culture filtrate, shows conclusively, so it seems, that deamination is brought about by living bacteria. It is a step preparatory to the utilization of nitrogenous constituents of cultural mediums for energy. The absence of ammonia from corresponding cultures containing sufficient dextrose is additional evidence: here the bacteria derive their energy from the carbohydrate, utilizing the nitrogenous constituents chiefly for their structural needs. Ammonia, therefore, as such, is a waste product, not a structural element.<sup>23</sup>

#### SIGNIFICANCE OF BACTERIAL METABOLISM

*In the Study of Intestinal Bacteriology.*—It is a well attested fact, known since Escherich's classic studies on the subject,<sup>24</sup> that the bacterial intestinal flora of the normal nursling is essentially fermentative: proteolytic bacteria and proteolysis alike are absent. As the infant becomes older and the diet more varied, the character of this intestinal flora undergoes a marked change: the fermentative organisms, *B. bifidus*, *B. acidophilus* and *Micrococcus ovalis*, tend to disappear, and organisms, as *B. coli*, that can accommodate their metabolism to presence or absence of carbohydrate take their place. That this microbic change parallels the dietary change appears to be rather generally agreed on, but few observations are recorded which give a fairly definite picture of the comparative chemical changes induced by intestinal bacteria of nurslings, artificially fed babies, adolescents and adults. Table 9, abbreviated from tables previously published,<sup>25</sup> shows the changes induced in the nitrogenous constituents of cultural mediums, similar to those already studied in the preceding tables, when they are respectively inoculated with the mixed intestinal bacteria from a normal nursling, a normal artificially fed infant, a normal adult and an adult having a heavy intestinal overgrowth of *B. welchii*.

The salient analytical details are shown for the normal nursling, the artificially fed infant, the normal adult and the adult with gas bacillus overgrowth. It will be seen that the conditions of experiment here are in a measure the reverse of a feeding experiment in man in that it is the mixed intestinal bacteria that are fed on selected diets.

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23. See Sears and Gourley: J. Bact. **15**:357, 1928.

24. Escherich: Darmbakterien des Säuglings, Stuttgart, 1886.

25. Kendall; Day, and Walker: Chemistry of the Intestinal Flora of Nurslings, J. Infect. Dis. **38**:200, 1926; Chemistry of the Intestinal Bacteria of Artificially Fed Infants, *ibid.*, p. 205; Chemistry of the Intestinal Flora of Normal Adults, *ibid.*, p. 211; Chemistry of the Intestinal Flora of Man Containing Abnormal Numbers of Gas Bacilli, *ibid.*, p. 217.

It must be borne in mind in these experiments with the intestinal flora, that a considerable variety of types of bacteria are, or may be, present in each inoculum. It follows that the chemical changes elicited by the growth of these mixtures of bacteria connote a resultant totality of reaction which may be greater in amount than, and even different in character from, the sum of the activities of the several kinds of bacteria growing in pure culture.<sup>26</sup> This has been shown, for example, in the associative action of certain strains of *B. coli* and *B. mesentericus*.<sup>27</sup> Growing separately in pure culture in milk, *B. coli* causes coagulation, and *B. mesentericus*, digestion. Grown symbiotically, they induce a stormy fermentation closely simulating that of *B. welchii*. Nevertheless, as is clearly shown in table 8, the several distinct types of intestinal flora, approach, both qualitatively and quantitatively, one or another of the types of chemical activity described in previous sections. The origin and significance of these several manifestations of chemical activity have been explained at length; therefore, only the more general phenomena will be discussed in association with the respective types of intestinal flora here.

Normal Nursling (table 9): The mixed intestinal flora from the normal breast-fed infant is characteristically fermentative. There is complete absence of putrefactive bacteria, and there is no evidence of proteolytic activity. In the sugar-free medium, however, there is slight deamination, as compared with the corresponding growth in the dextrose medium. The small amount of ammonia produced is indicative of a preponderance of *M. ovalis*, rather than of *B. acidophilus* or of *B. bifidus* in the culture. *M. ovalis* grows better in mediums freed from carbohydrates than either of the bacilli.<sup>28</sup> Smears made from the plain and the dextrose mediums show few *acidophili* and *bifidi* in the former, but many *bifidi* in the latter. *M. ovalis* is predominant in the former and abundant in the latter. The increase in protein nitrogen, with a concomitant decrease in polypeptid nitrogen, most marked on the sixth day of incubation, is in accord with previous observations. The significance of the obligately fermentative and lactacidogenic intestinal flora, the natural defensive barrier of the breast-fed infant against microbic intestinal infectants, has been recognized ever since Escherich's classic study of the bacteria of nurslings.<sup>24</sup>

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26. Castellani and Chalmers: Brit. M. J. **2**:855, 1917; **1**:183, 1919. Holman: J. Infect. Dis. **39**:145, 1926. Sears and Putnam: *ibid.* **32**:270, 1923. Kendall (footnote 4, p. 78).

27. Kendall: Boston M. & S. J. **163**:322, 1910.

28. Kendall and Haner: Metabolism of *Micrococcus Ovalis*, *B. Acidophilus*, *B. Bifidus*, J. Infect. Dis. **35**:67, 77 and 89, 1924.

Normal Artificially Fed Infants: The outstanding chemical feature of the metabolism of the intestinal flora of the artificially fed infant lies in the striking contrast between the considerable nitrogenous changes induced by the mixed flora in the sugar-free medium and the very small nitrogenous changes induced in the corresponding sugar-containing

TABLE 9.—*Changes Induced in the Nitrogenous Constituents of Cultural Mediums, When These Are Inoculated with the Mixed Intestinal Bacteria from Normal Nursling, Normal Artificially Fed Infant, Normal Adult and Adult with Overgrowth of Gas Bacillus*

Nitrogenous Constituent	Results of Analyses, Mg. per 100 Cc. of Medium									
	Plain Medium					Dextrose Medium				
	Con- trol	1 Day	3 Days	6 Days	9 Days	Con- trol	1 Day	3 Days	6 Days	9 Days
<b>Normal nursling</b>										
Total nitrogen.....	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein nitrogen.....	770	780	770	790	770	790	790	790	800	792
Nonprotein nitrogen...	230	220	230	207	231	210	210	210	200	208
Polypeptid nitrogen...	173	155	156	120	134	152	151	153	138	150
Aminonitrogen.....	36	32	32	38	41	35	30	28	34	34
Ammonia nitrogen....	21	33	42	47	55	22	27	26	24	22
<b>Artificially fed infant</b>										
Total nitrogen.....	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein nitrogen....	770	760	690	680	675	770	780	760	760	770
Nonprotein nitrogen...	229	240	310	321	324	229	219	240	240	230
Polypeptid nitrogen...	182	168	213	179	164	182	169	184	186	163
Aminonitrogen.....	33	39	20	22	29	33	35	32	32	42
Ammonia nitrogen....	14	33	89	123	131	14	16	15	15	23
<b>Normal adult</b>										
Total nitrogen.....	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein nitrogen.....	816	803	796	792	778	828	828	820	829	828
Nonprotein nitrogen...	183	195	204	206	216	171	182	182	171	171
Polypeptid nitrogen...	131	131	127	83	72	121	129	127	113	111
Aminonitrogen.....	30	35	30	31	36	30	30	30	32	34
Ammonia nitrogen....	21	28	48	91	109	20	24	26	26	26
<b>Adult with excessive num- ber of gas bacilli</b>										
Total nitrogen.....	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein nitrogen.....	816	791	604	515	450	816	816	805	805	828
Nonprotein nitrogen...	182	208	390	488	550	183	183	195	195	171
Polypeptid nitrogen...	124	126	220	226	232	129	143	155	155	113
Aminonitrogen.....	38	44	37	43	25	33	19	18	16	34
Ammonia nitrogen....	20	37	134	219	270	20	22	23	24	24

medium. In the former, a reduction of the protein nitrogen fraction of about 90 mg. and of aminonitrogen of 10 mg. is rather more than compensated for by an increase of ammonia nitrogen amounting in the aggregate to 109 mg. It is clear that this shifting of nitrogenous constituents is not due to a proteolytic enzyme, else the polypeptid nitrogen would increase proportionally. The organism responsible for this type of reaction is a strong deaminizer. It appears to be quite similar to that described in another study (organism 62).<sup>29</sup> In the corresponding dextrose medium, the nitrogenous changes are very small indeed: there

29. Kendall; Day; Walker, and Haner: Bacteriology and Chemistry of Adult Duodenal Contents, J. Infect. Dis. 40:677, 1927.

is no evidence of proteolysis or of putrefaction. In this respect the chemical change is reminiscent of that induced by the intestinal flora of the normal nursling. The effects of dextrose in reducing the action of the bacteria on the nitrogenous constituents of the medium for energy needs are clearly shown. There is no evidence of deamination, proteolysis or putrefaction. The very small nitrogenous changes are those attributable to the usual building up of polypeptid nitrogen to protein nitrogen in the bodies of the growing bacteria.

**Normal Adult:** The changes induced by the mixed intestinal flora of the normal adult are not very different from those just described for the artificially fed baby. There is evidence of some proteolysis in the sugar-free medium. This is indicated by a small decrease in the protein nitrogen fraction and also by much deamination. The gain in ammonia nitrogen is some 70 mg.; it is less than that observed in the sugar-free cultivation of the mixed flora of the artificially fed infant, but materially greater than that characteristic of the normal nursling. In presence of dextrose, the chemistry of the flora of the normal adult reverts to the typical fermentative pattern characteristic of the several floras just described, as well as of those of the dysentery, typhoid, colon and *proteus* bacilli. There is no significant deamination or evidence of proteolysis. The significant nitrogenous change is a transfer of polypeptid nitrogen to protein nitrogen, consonant with the growth of bacteria in the medium.

**Adults with Excessive Number of Gas Bacilli:** The evidence deduced thus far from the chemical study of the mixed intestinal floras of nurslings, babies fed by bottle and normal adults has brought to light two principal facts. In the first place, in presence of carbohydrate, all these bacteria are reduced chemically to one common level—that of producers of lactic acid—in which state the bacteria have minimal action on the nitrogenous constituents of these mediums. In the second place, the mixed intestinal flora of the breast-fed infant is nonproteolytic and nonputrefactive, even in absence of utilizable carbohydrate, whereas the floras of the artificially nourished infant and of the normal adults, in absence of utilizable carbohydrate, are more or less proteolytic and putrefactive, as shown by the concomitant changes in the nitrogenous constituents of these cultural mediums. For purposes of comparison, the chemistry of mixed intestinal bacteria from an adult with an overgrowth of gas bacilli in the intestinal tract is introduced at this point. The salient details appear in table 9. The most striking chemical change is found in the ammonia fraction, which by the ninth day has increased to the surprising amount of 250 mg. This is accompanied by a decrease of 366 mg. in the protein nitrogen, indicating active proteolysis, and a gain of 128 mg. of polypeptid nitrogen, which, with the gain in

ammonia, accounts for almost the entire loss in protein nitrogen. It is evident, from what is known of the chemistry of *B. welchii* (the gas bacillus)<sup>30</sup> that the proteolytic changes are not due to the Welch bacillus, but to associated bacteria, among which nonfermenters of dextrose, as *B. alcaligenes* and the one found in the flora of the artificially fed baby, are usually prominent. Here again is an example of symbiotic action, in which two bacteria, working together, produce unusual chemical changes. In sharpest contrast with the deamination found in the sugar-free medium is the utter absence of more than minimal nitrogenous changes in the corresponding dextrose-containing culture. Here there is the customary increase in protein nitrogen with its concomitant decrease in polypeptid nitrogen. Deamination is reduced almost to zero. A word of caution must be injected here, however. The addition of carbohydrate to the diet of the patient whose fecal bacteria are under consideration would be very harmful. If this were done, the gas bacilli would go on a rampage, with consequences very distressing to the victim of such ill-advised dietary treatment. Well soured milk is indicated in such cases. The gas bacilli are thereby frustrated in their growth, and the patient is benefited.<sup>31</sup> The results, in terms of chemical change on a diet rich in sour milk, would be very similar to those achieved by the addition of dextrose to the culture medium. Deamination and indications of proteolysis would be reduced to the vanishing point, while at the same time the activity of the gas bacilli would be curbed.

*In Clinical Practice.*—Several important clinical applications of this sparing action of carbohydrates for nitrogenous substances readily suggest themselves, as follows:

Reduction of Intestinal Putrefaction: It is generally believed that urinary indican is an indication of intestinal putrefactive processes of microbic causation. This is in the main true. However, a much more important substance is overlooked in the ordinary tests, namely, indol. Indoluria, not indicanuria, has the greater clinical significance, even though both substances have a common point of origin in intestinal putrefaction. Under ordinary circumstances, indol is oxidized in the liver to indoxyl, then paired, and excreted as indoxyl sodium sulphonate. This is urinary indican. If, however, this function of the liver is interfered with and oxidation of indol does not take place, indol, not indican, may appear in the urine. This may also happen if the amount of indol

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30. Kendall; Day, and Walker: J. Infect. Dis. **30**:141, 1922.

31. For clinical and chemical aspects of gas bacillus overgrowth in the intestinal tract of man, see Kendall: Intestinal Intolerance for Carbohydrate Associated with Overgrowth of Gas Bacillus (*Bacillus Welchii*), J. A. M. A. **86**:737, 1926; Kendall and Varney: J. Infect. Dis. **41**:143, 1927; Kendall and Gebauer: *ibid.* **47**:261, 1930.

is temporarily greater than the liver can manage. Indoluria, therefore, is, or may be, an indication of impaired function of the liver, as well as of intestinal putrefaction. The formation of indol in the urine, as has been indicated, may be greatly restricted by suitable dietary procedure.

**Dietary Treatment in Typhoid Fever and Dysentery:** The effects of diets high in carbohydrate in typhoid fever were studied by Coleman and Shaffer<sup>32</sup> from a purely chemical point of view. They found that the loss of weight was materially reduced, and the general condition of the patient materially improved when large daily feedings of lactose were given, and were disposed to attribute these gains to the well known dictum that "carbohydrate spares body protein." An additional and highly important factor seems to have been overlooked, however. The diet high in lactose has a profound effect on the metabolism of the typhoid bacillus itself. In presence of carbohydrate this microbe, of course, becomes a producer of lactic acid. Herein, probably lies the otherwise unexplainable reduction in toxemia which Coleman and Shaffer noticed when their patients were placed on the diet high in calories. Under these circumstances the typhoid bacilli, in virtue of their altered metabolism (Dr. Jekyll and Mr. Hyde again), utilize carbohydrate for energy in preference to protein. The first deliberate attempts to utilize the sparing action of carbohydrates for proteins with its associated change in the products of metabolism in the treatment of disease were made in Boston in 1911 and subsequent years.<sup>33</sup> The results were satisfactory in cases of bacillary dysentery and in gas bacillus diarrheas. The same procedure was subsequently used by Torrey<sup>34</sup> in his studies on the effects of diet in typhoid fever. The importance of these dietary adjuvants in the field of clinical medicine is gradually being recognized.<sup>35</sup>

**Production of Soluble Specific Toxins by Bacteria:** An important example of the sparing action of carbohydrate for protein, which is an essential factor in the commercial production of specific antitoxin, was first described by Theobald Smith<sup>36</sup> many years ago, before much was known of the chemistry of bacteria. He found that diphtheria bacilli do not yield toxin in presence of more than minimal amounts of

32. Coleman and Shaffer: Protein Metabolism in Typhoid Fever, *Arch. Int. Med.* **4**:538, 1909.

33. Kendall: *Boston M. & S. J.* **164**:288, 1911; Kendall and Smith: *ibid.*, p. 306.

34. Torrey: *J. Infect. Dis.* **16**:72, 1915.

35. Stokes, J. H.: The Effect on the Skin of Emotional and Nervous State: III. Theoretical and Practical Consideration of a Gastro-Intestinal Mechanism, *Arch. Dermat. & Syph.* **22**:962, 1930.

36. Smith, Theobald: *J. Exper. Med.* **4**:373, 1899.



dextrose. It is now known that in presence of dextrose the diphtheria bacillus forms lactic acid in place of toxin.<sup>37</sup> As potent toxin is essential for the production of antitoxin, this principle of reducing utilizable sugar in mediums for the production of toxin is rigorously adhered to. It should be stated that the same phenomenon is met with in the preparation of the toxin of *B. welchii*<sup>38</sup> and others.

"Resting Bacteria" in Vaccine Therapy: A word should be said at this time about the newly explored field of "resting bacteria." The significant pioneer work was done largely by Quastel<sup>39</sup> and his associates, between the years 1924 and 1928. He investigated several problems of significance to the understanding of microbic action by means of these "resting" organisms.

"Resting bacteria" are actively growing organisms harvested at the time at which they are proliferating most actively. After careful washing by centrifugation, repeated several times to insure removal of all alien material, they are kept in suspension in salt solution at the temperature of the icebox for several days without material impairment of their original activities. These "resting" bacteria "initiate changes in substrates, which they, as proliferating bacteria, would subsequently use for their energy requirements."<sup>40</sup> The amount of chemical changes such bacteria can bring about in a very few hours is remarkable.<sup>41</sup> One very suggestive clinical application of "resting" bacteria has arisen in connection with vaccine therapy. It has been found<sup>42</sup> that bacteria harvested as described and therefore freed from adhering cultural medium, etc., when killed by exposure to tenth-normal formaldehyde solution (neutral in reaction) for two hours, are much more effective in the treatment of furuncles and similar focal infections. There is also considerable evidence that pneumococci, prepared in similar manner after growth on blood agar, have materially greater antigenic and protective power than can be had from cultures of the same types not carefully washed from adherent products of growth and constituents of cultural medium. This aspect of bacteriology is still very immature, but it holds forth promise of many important contributions which will help fill in some of the great gaps in present knowledge of the mechanisms of growth in general and of microbic infection in particular.

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37. For chemical evidence, see Kendall; Day, and Walker: *J. Am. Chem. Soc.* **35**:1210, 1913; **36**:1950, 1914.

38. Bull and Pritchett: *J. Exper. Med.* **26**:603 and 687, 1917.

39. Quastel: *Biochem J.*, 1924-1927, vols. 18-21; for brief summary, see *J. Hyg.* **28**:139, 1928.

40. Kendall and Ishikawa: *J. Infect. Dis.* **44**:282, 1929.

41. Kendall; Ishikawa, and Friedemann: *J. Infect. Dis.* **47**:186, 1930.

42. Details will be published soon.

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, etc.**—Stanhope Bayne-Jones, professor of bacteriology in the school of medicine and dentistry of the University of Rochester, has been appointed master of college and professor of bacteriology in the school of medicine of Yale University.

Kenneth M. Lynch, professor of pathology in the Medical College of the State of South Carolina, has been given the honorary degree of LL.D. by the University of South Carolina.

Carroll Gideon Bull, professor of immunology in the school of hygiene and public health of the Johns Hopkins University, has died.

Charles Phillips, has resigned as professor of pathology in the Medical College of Virginia in Richmond to assume the directorship of the laboratories of the Scott and White Clinic, Temple, Texas.

Herbert H. Waite, professor of bacteriology at the University of Nebraska, Lincoln, has died at the age of 62.

Ralph McBurney has been appointed professor of bacteriology and hygiene in the University of Alabama.

Axel Holst, professor of bacteriology and hygiene in the University of Oslo and well known for his pioneer investigations in deficiency diseases, has died at the age of 70.

Emmerich von Haam, associate professor of pathology in the school of medicine in the University of Arkansas, has been appointed assistant professor of pathology and bacteriology in the State of Louisiana Medical Center, New Orleans.

Shibamiro Kitasato, celebrated pioneer Japanese bacteriologist, has died at the age of 72. He was a pupil of Robert Koch. He first grew the tetanus bacillus in pure culture; he was the co-discoverer with Behring of diphtheria and tetanus antitoxins in 1890; in 1894 he discovered the plaque bacillus at about the time that it was discovered independently by Yersin; he was director of the Imperial Institute for Infectious Diseases of Japan before he organized the Kitasato Institute for Infectious Diseases in Tokyo.

Alvin G. Foord, pathologist in the Buffalo General Hospital, has been appointed pathologist in the Pasadena Hospital, Pasadena, Calif.

**Society News.**—At the recent meeting of the American Medical Association in Philadelphia gold medals were awarded to Jacob Furth of the Henry Phipps Institute of the University of Pennsylvania, for his work on experimental leukemia in the mouse and the fowl, and to J. Parsons Schaeffer and Warren B. Davis, Jefferson Medical College, for anatomic studies on the nasal sinuses. Silver medals were awarded to Bedford Shelmire, Dallas, Texas, and to W. E. Dove, U. S. Bureau of Entomology for their work on the spread of typhus fever by the tropical rat mite, and to Harrison S. Martland, A. O. St. George, A. O. Gettler and R. H. Mueller for demonstration of industrial radium poisoning.

# Abstracts from Current Literature

## Experimental Pathology and Pathologic Physiology

OSSIFICATION: I. CALLUS FORMATION AND CALCIFICATION. I. NEWTON KUGELMASS and RICHARD N. BERG, *Am. J. Dis. Child.* **41**:236, 1931.

The relation between callus formation and the amount and rate of calcification was studied in rabbits. The greater the local formation of fibrous tissue, the greater was the amount and degree of calcification, all other conditions remaining the same. The blood calcium tends to be higher and the phosphate lower in the course of normal bone repair, and the more rapid the rate of calcification the more marked is this alteration in the calcium and phosphate content of the blood. Injection of an alkaline solution of trypsin, at the site of fracture to produce fibrous dissolution, markedly retarded the union of bones in comparison with that in the control series. Injection of a solution of tissue fibrinogen at the site of fracture, to stimulate increased callus formation, markedly accelerated the union of bones in comparison with that in the control series. The maintenance of animals with induced fractures on a diet high in protein, to increase the blood-clotting factors, produced no local effect in bone repair as compared with the control series. Ossification is a local transformation of fibrous tissue into bone, the constituents of which are derived from the blood. Bone repair is a local rather than a systemic process, and union may therefore be induced or accelerated by local measures.

AUTHORS' SUMMARY.

MONGOLISM IN ONE OF TWINS. J. I. WARING, *Am. J. Dis. Child.* **41**:351, 1931.

This case of mongolism in one of dizygotic twins supports the view that the condition is the result of a defect in germ plasm and not due to the general physical condition of the parents.

AUTHOR'S SUMMARY.

SPLENOMEGALY AND HEPATIC ENLARGEMENT IN HEREDITARY HEMORRHAGIC TELANGIECTASIA. THOMAS FITZ-HUGH, JR., *Am. J. M. Sc.* **181**:261, 1931.

Four cases of hereditary hemorrhagic telangiectasis are reported, which exhibited the following noteworthy features: splenomegaly and hepatic enlargement; increasing intolerance to transfusions of blood, resulting in posttransfusion jaundice (and death in two); the coincidence of identical blood group (0) in all of the patients tested who presented the splenomegalic syndrome.

AUTHOR'S SUMMARY.

HYPERTENSION IN CASES OF CONGENITAL POLYCYSTIC KIDNEY. FREDERICK W. SCHACHT, *Arch. Int. Med.* **47**:500, 1931.

Evidence at hand indicates that in the majority of cases of polycystic kidney, significant or persistent hypertension is present. There is marked thickening of the walls of the arterioles and of the small arteries of the polycystic kidney, with consequent reduction in the ratio between the thickness of the wall and the diameter of the lumina of the vessels of the kidney. The high incidence of retinal sclerosis in these cases indicates that the process probably is associated with a generalized vascular disturbance.

AUTHOR'S SUMMARY.

TUMOR OF THE BRAIN WITH DISTURBANCE IN TEMPERATURE REGULATION. ISRAEL STRAUSS and JOSEPH H. GLOBUS, *Arch. Neurol. & Psychiat.* **25**:506, 1931.

Strauss and Globus report three cases which they believe support facts in favor of the existence of a heat-regulating center in the hypothalamus. The first case

presented a rather indefinite and inconstant clinical picture of mental symptoms (confusion and disorientation) with neurologic signs—changes in the reflexes and gait and urinary disturbances. The only sign that was fairly constant was an elevated temperature of 102 F. At necropsy, multiple tumors (spongioblastomas) of the corpus callosum and septum pellucidum were discovered with invasion of the anterior horn of the left lateral ventricle, of the walls of the third ventricle and practically its entire floor.

In the second case, that of a man, aged 62, there was also a mixture of mental and neurologic signs and symptoms with an unaccountable elevation of temperature (from 102 to 103 F.). Necropsy, which followed a decompression operation, showed multiple tumors. One tumor (a spongioblastoma) was situated in the region of the right optic thalamus and hypothalamus, which were invaded from the walls of the third ventricle and extended as far as the tuber cinereum below and as high as the taeniae thalami above. Another tumor was situated in the third temporal convolution of the left hemisphere.

In the third case, that of a woman, aged 57, in which the prominent symptoms were sleepiness and mental derangement associated with an elevation of temperature (103 F.) and a slow pulse, a diagnosis of tumor of the third ventricle was made, which was substantiated by necropsy (a glioma in the region of the optic chiasm).

An important general conclusion from the study of the three cases seems to be justified that a tumor of the periventricular zone of the third ventricle and in the tuber cinereum causes "a disturbance in the function of the heat-regulating mechanism."

GEORGE B. HASSIN.

THE BRAIN AND THE CEREBROSPINAL FLUID IN EXPERIMENTAL CEREBRAL EMBOLISM. WILLIAM CONE and S. E. BARRERA, *Arch. Neurol. & Psychiat.* **25:523**, 1931.

By injecting cut up black silk suture material, poppy seed and paraffin melting at 45 C. into the carotid the authors produced aseptic emboli in the brains of dogs. The animals were allowed to survive for variable periods up to seven days. Before they were killed, a cisternal puncture was performed. Twenty dogs that showed clinical signs of cerebral embolism were killed from eight and one-half to ninety-six hours after operation; one was killed after seven days. Small branches of the middle cerebral artery were usually blocked, causing areas of softening in the basal ganglions or the ependyma and the adjacent tissues. The spinal fluid cells were studied according to the method of Alzheimer. Polymorphonuclear neutrophilic leukocytes were present in the perivascular spaces of the infarcted areas and in the meninges (over the embolic area); the number of leukocytes increased during successive days, reaching its height at the end of forty-eight hours. After this time, they degenerated. They disappeared after seven days, granule cells replacing them in the softened area. A remarkable phenomenon was evident: The perivascular spaces of the cerebral blood vessels, filled with polymorphonuclear leukocytes, were seen plunging into the subarachnoid spaces. Organisms were not present in any of the animals. The astrocytes were badly damaged; their perivascular feet were broken up, thus disrupting the glial barrier and permitting the invasion of the cerebral parenchyma by mesodermal, polymorphonuclear elements.

In human material, polymorphonuclear cells were also numerous in the infarcted areas (within five and one-half days after the apoplexy), and in each instance they were present in the leptomeninges.

GEORGE B. HASSIN.

THE INFLUENCE OF TESTICLE EXTRACT ON THE INTRADERMAL SPREAD OF INJECTED FLUIDS AND PARTICLES. D. C. HOFFMAN and F. DURAN-REYNALS, *J. Exper. Med.* **53:387**, 1931.

The experiments in this paper show that testicle extract causes particles of india ink and of Prussian blue to spread much more extensively through the

intercellular spaces than similar suspensions made with Ringer's solution. Methylene blue (methylthionine chloride, U. S. P.) inoculated intravenously localizes more extensively in areas receiving injections of tissue extracts than in control areas receiving injections of tissue extracts without enhancing power. Kidney extracts have this property to a less degree, whereas spleen extracts and blood serum are devoid of it. The spreading power of extracts is destroyed by heating at 60 C. for thirty minutes, as is also the power to enhance infections. The precise mode of action of the Reynals factor is not known, but the results of the experiments here presented suggest that it may depend at least in part on the property whereby testicle extract increases the spread of injected material and alters the permeability of tissue cells. It is not inconceivable that changes in permeability facilitate the passage of vaccine virus through the endothelial cells of the blood and lymph vessels, and lead to the generalized vaccinia which is of frequent occurrence in the reported results. It has been shown that fluids and suspensions of inert particles are spread by the extract. *B. tetanus* and *B. coli* exotoxins and trypsin were not enhanced in their action despite the fact that they were spread through a more extensive area in the tissues. Viruses, on the other hand, are markedly influenced and in this respect resemble bacteria, not toxins and enzymes. It appears probable that a definite capacity for multiplication on the part of an injected substance is required if its pathogenic effects are to be enhanced. It may be concluded tentatively that the enhancing power of the testicle extract may depend on that property which not only spreads the injected material through a larger area but renders the tissue cells more easily penetrable by the agents.

AUTHORS' SUMMARY.

THE SKIN REACTIONS PRODUCED BY ALTERNATIONS OF HEAT AND X-RAY AT VARIOUS TIME INTERVALS. J. A. HAWKINS, J. Exper. Med. **53**:405, 1931.

Areas on the abdomen of the same guinea-pig were exposed to suberythreal doses of soft x-rays, to heat of an intensity below the critical dose for the production of burns and to both radiations in sequence with various time intervals between the exposures. The only effect of exposure to x-ray or heat alone was a slight scaling of the skin. The areas exposed to heat and roentgen radiation developed well marked and persistent burns when the exposure to one agent was made within three hours of the other. Scaling of the skin developed when the exposure to one agent was made one day after the other. This scaling was more marked and lasted longer than the scaling produced by either agent alone. The results were the same no matter in which sequence the agents were applied.

AUTHOR'S SUMMARY.

EFFECTS OF THE INTRAVENOUS INJECTION OF COLLOIDAL SILVER UPON THE HEMATOPOIETIC SYSTEM IN DOGS. S. S. SHOUSE and G. H. WHIPPLE, J. Exper. Med. **53**:413, 1931.

Colloidal silver has no specific action on the bone marrow of dogs but is a systemic poison which may cause anorexia, weakness, loss of weight, anemia and death. Hemolysis can be demonstrated after the administration of large doses of colloidal silver, and the anemia presumably is due, in part at least, to a destruction of red blood cells in the peripheral circulation. The colloidal silver, injected intravenously, is deposited as granules almost exclusively in the cells of the reticulo-endothelial system, after the manner of particulate substances. Repeated injections of nonlethal amounts of this substance are invariably followed by hyperplasia of the bone marrow. In no case was aplasia found. Large single doses of this material cause rapid death in twelve hours or less, characterized by pulmonary edema and congestion. An initial increase in the number of erythrocytes and leukocytes may occur following the use of smaller amounts of silver, but repeated injections cause considerable anemia, without a definite increase in the leukocytes and with no signs of a deficiency in blood platelets.

AUTHORS' SUMMARY.

APLASIA OF MARROW AND FATAL INTOXICATION IN DOGS PRODUCED BY ROENTGEN RADIATION OF ALL BONES. S. S. SHOUSE, S. L. WARREN and G. H. WHIPPLE, *J. Exper. Med.* **53**:421, 1931.

Constant results were obtained in the acute reaction to the specified amount of heavily filtered radiation over the bony skeleton. On the eighth or ninth day after exposure to the radiation, a short and fatal intoxication develops without warning. A profound leukopenia appears after from five to six days and is maintained in the peripheral blood (200 white blood cells or less per cubic millimeter) for the two or three days before death. The platelets suddenly disappear from the blood smears the day before death. This has some bearing on the life cycle of the platelet. All of the organs and structures of the body present extensive generalized capillary hemorrhage of recent origin. The substance of the spleen and lymph nodes is greatly reduced, and the germinal centers are visible only as remnants. The red cell hematocrit reading drops from about 50 per cent, or normal, to approximately 40 per cent. The bone marrow is depleted of all its cells except the connective tissue and fat cells, blood vessel endothelium, phagocytes filled with brown granules and occasional normoblasts.

AUTHORS' SUMMARY.

THE COMBINED EFFECTS OF COLLOIDAL SILVER AND HIGHLY FILTERED ROENTGEN RADIATION UPON THE HEMATOPOIETIC SYSTEM IN DOGS. S. S. SHOUSE and S. L. WARREN, *J. Exper. Med.* **53**:437, 1931.

The individual destructive effects of colloidal silver and heavily filtered radiation are still evident when the two are used together. The combined effects are cumulative in that small doses are more destructive than when either is used alone. The leukocytosis resulting from the injection of the colloidal silver affords no protection against the terminal leukopenia following the irradiation.

AUTHORS' SUMMARY.

THE TOXICITY OF CERTAIN NORMAL SERA FOR THE GUINEA PIG. M. E. FIELD, *J. Immunol.* **20**:89, 1931.

Lethal toxicity of human and rabbit serum persists for at least seventy-two hours. This toxicity decreases with age, so that serum one week old is usually completely innocuous. Primarily toxic normal serum causes a marked rise in the pulmonary arterial pressure, accompanied by bronchoconstriction. Nonagglutinating serum acts in every way as does normal serum. The cellular extract of hemolyzed guinea-pig cells is very toxic for the guinea-pig, causing a great rise in the pulmonary arterial pressure, followed by death. Toxic serum causes great intra vitam hemolysis which decreases in amount as the toxicity of the serum is attenuated by time.

AUTHOR'S SUMMARY.

FIBRINOPENIA. R. JÜRGENS and H. TRAUTWEIN, *Deutsches Arch. f. klin. Med.* **169**:28, 1930.

Jürgens and Trautwein review the three cases of hemorrhagic diathesis with complete or almost complete disappearance of the fibrinogen from the blood reported in the literature and to these add the clinical history of a fourth case, the first one ever observed in an adult. Examination of the blood revealed a great deficiency of fibrinogen but a normal amount of all the other components of coagulation. Postmortem examination disclosed an almost completely isolated, extensive carcinosis of the bone with fibrous transformation of the bone marrow. The primary growth was a carcinoma of the prostate. The macroscopic and particularly the microscopic changes indicated that the bone marrow plays a part in the formation of fibrinogen.

## Pathologic Anatomy

IS FAMILIAL JAUNDICE OF NEW-BORN INFANTS ERYTHROBLASTOSIS? WILLIAM L. BUHRMAN and HEYWORTH N. SANFORD, *Am. J. Dis. Child.* **41**:225, 1931.

Two cases of fatal jaundice in new-born infants are reported. Both cases showed a familial history of jaundice at birth. The clinical picture was one of familial jaundice with deep progressive icterus, enlarged liver and spleen, bile pigments in the urine and an indirect van den Bergh reaction. Both cases were characterized by an intense anemia with extremely numerous nucleated red cells. In one case the time of bleeding was greatly increased and there was a reduction in the blood platelets. Both patients died, and autopsy showed erythropoiesis, blood-forming islands in the liver and hyperplasia of the bone marrow.

AUTHORS' SUMMARY.

THE INVOLVEMENT OF THE CORONARY ARTERIES IN ACUTE RHEUMATIC FEVER. S. R. SLATER, *Am. J. M. Sc.* **181**:203, 1931.

Three cases of rheumatic coronary arteritis with closure are reported. The symptomatology and the prognosis are discussed.

AUTHOR'S SUMMARY.

PANCREATITIS COMPLICATING MUMPS. M. B. BRAHDY and I. H. SCHEFFER, *Am. J. M. Sc.* **181**:255, 1931.

Pancreatitis complicating mumps produces a characteristic symptom-complex. It occurs in both sexes and at any age. In our series the extremes of age were 6 and 30 years. The onset is sudden between the fifth and eleventh days of the illness with nausea, anorexia and fever. The temperature rises in a day or two to 103 or 104 F. in the moderately severe cases, and returns to normal in from three to five days. The pulse is usually slow in relation to the temperature. The patients complain of malaise, nausea, headache and abdominal pain, and usually vomit. The pain and tenderness in our cases were always in the upper part of the abdomen. Most of our patients were constipated, and diarrhea, which has been reported by other observers, did not occur in our series. There was no glycosuria. Information from autopsy examinations is limited in two cases. The pathologic changes in the pancreas reported by Lemoine and Lapasset are comparable to the changes found by Wollstein in the parotid glands of monkeys experimentally inoculated with mumps. The abnormal findings in the pancreas described by the French authors are in all probability only a little more advanced than those which occur in the patients who recover.

AUTHORS' SUMMARY.

DIFFUSE PROGRESSIVE DEGENERATION OF THE GRAY MATTER. BERNARD J. ALPERS, *Arch. Neurol. & Psychiat.* **25**:469, 1931.

In a carefully studied brain from a 4 months old infant which had had convulsions, restlessness, frozen pupils and practically no other physical neurologic findings, Alpers found a large number of pathologic conditions. They were present mainly in the cortex, basal ganglions and pons, and were classified as developmental and structural. In the former he included paucity of ganglion cells in the third cortical layer of the frontal, parietal and occipital lobes; accumulation of foci of embryonic cells (probably ependymal spongioblasts) underneath the ependyma, mostly of the frontal parts of the brain and one large focus of such cells in the parolfactory area; an immature state of the macroglia and ganglion cells some of which had a moth-eaten cytoplasm; demyelination of the cortex, corpus striatum and optic thalami with immense masses of blood vessels and capillaries many of which were budding. An interesting feature was an abundance of ganglion cells in the white matter of the brain and the cerebellum. The subependymal region was thickly covered with masses of spongioblasts, some of

which were rather remote from the ependyma. In addition there were evidences of arteriosclerosis in a pial blood vessel.

The structural changes were as foci of softening or necrosis, mostly of atypical nature, incomplete and confined to the cortex and the basal ganglions. The optic thalamus contained a typical focus with gitter cells and a marked mesodermal reaction which was absent in the atypical foci. In general, the reaction to the vast parenchymatous lesion of the brain was neuroglial, not mesodermal. The corpus striatum and the walls of the blood vessels contained lipoids. Alpers sums up his observations as an incomplete necrosis of the brain described by Jakobs. He quotes two more similar cases studied in the latter's laboratory and contrasts them with the diffuse periaxile sclerosis of Schilder where the white matter is principally affected, while in the three cases from Jakob's laboratory the process affected mainly the gray matter. The problematic cause of the cortical destruction is likely an intoxication.

GEORGE B. HASSIN.

DISSEMINATED ENCEPHALOMYELITIS. ROY R. GRINKER and PETER BASSOE, Arch. Neurol. & Psychiat. **25**:723, 1931.

The authors report four cases of a diffuse involvement of the cerebrospinal nerve with the main view to a broad pathologic classification of various types of encephalitis. In the first patient, five days after a respiratory infection and bulbar symptoms and symptoms of the spinal cord developed. Death occurred from pneumonia. The pia-arachnoid showed lymphocytes mixed with granule cells, the blood vessels were engorged; the parenchyma of the brain and spinal cord, and especially the white matter of the medulla and the sacral portion of the spinal cord, was covered with foci of softening situated around the blood vessels (mostly veins); lymphocytes and plasma cells were also seen around the blood vessels, but fat-filled cells were much more numerous. The authors consider this case not one of disseminated encephalomyelitis, but rather of the same type as cowpox encephalitis.

In case 2, a gradual hemiplegia developed in a child, aged 10. Death occurred twenty-three days after a progressive course and a temperature of 103 F. Microscopic examination revealed areas of demyelination in the subcortex, with many myelin fibers preserved and without relationship to the blood vessels. The latter showed granule cells (in the damaged areas, while in remote regions they exhibited hematogenous elements mainly). While the infectious character of the lesions is not denied by the authors, the toxic degenerative nature of the foci of softening is not readily admitted by them.

In case 3, in which the authors made the diagnosis of subacute disseminated encephalomyelitis, the duration was five months, after a sudden onset with vertigo, vomiting and diplopia. Three weeks later the acoustic, facial and right sixth nerves became involved, followed by ataxia in the right leg and arm and hemiparesis of the left side of the body. About one month before death, clonic spasms in the right arm appeared, followed by paralysis. The general condition grew much worse; difficulties of speech set in, with optic neuritis and bulbar phenomena followed by death. Necropsy revealed in the region of the pons, medulla and cerebral peduncles patches of demyelination. The affected areas contained fat scattered in the tissues and in the perivascular spaces of the adjacent blood vessels mixed with lymphocytes, but the patches had "no correlation with a definite arterial supply." Older patches contained fibroblasts and small amounts of reticulin.

In the fourth case, a staggering gait and bulbar symptoms with inability to control the right arm in writing developed in the patient two months after a febrile attack. The symptoms gradually increased within the next two years. Examination revealed diplopia, nasal speech, paralysis of the left vocal cord and left facial nerve (peripheral type) with a right hemiparesis. While the patient was in the hospital a febrile attack developed with aggravation of the general condition; the patient left unimproved. On the basis of three cases examined



pathologically a classification of various types of encephalomyelitis has been attempted. The greatest majority, including such disease processes as multiple sclerosis and Schilder's disease, cannot, according to the authors, be considered primarily degenerative, and the presence of hematogenous elements in the perivascular spaces should not be overestimated in diagnosing a case as due to an inflammatory process.

GEORGE B. HASSIN.

ENCEPHALITIS AND ENCEPHALOMYELITIS IN MEASLES. ARMANDO FERRARO and I. H. SCHEFFER, *Arch. Neurol. & Psychiat.* **25**:748, 1931.

Six cases of lesions of the central nervous system following measles have been studied. Though somewhat varying in severity, the changes were practically alike. They were: perivascular demyelination, though foci with loss of myelin were present away from the blood vessels, perivascular proliferation of microglia, mild and only occasionally hematogenous (lymphocytes, occasionally plasma cells) perivascular infiltrations, mild meningeal changes and parenchymatous changes varying from acute swelling of ganglion cells to their liquefaction. The vessels were engorged, sometimes thrombosed and proliferated. The macroglia showed both proliferative and regressive changes. The perivascular proliferation of the microglia was mainly in the white substance. The type of changes is classified as encephalopathy instead of encephalitis, because of the practical absence of hematogenous elements in the perivascular spaces. Whether the cause is a direct action of the virus of measles, a toxin or some allergic phenomenon is left unanswered.

GEORGE B. HASSIN.

CEREBELLAR HEMANGIOBLASTOMAS WITH INCIDENTAL CHANGES OF THE SPINAL CORD. C. DAVISON, W. SCHICK and S. P. GOODHART, *Arch. Neurol. & Psychiat.* **25**:783, 1931.

In the first patient, a woman, aged 42, diplopia developed thirteen years before admission to the hospital, accompanied by dizziness and tinnitus; one year later vision became impaired, while the diplopia was intermittent. About three years before admission, the patient had difficulties in walking and urinary disturbances and vomited. Examination at this time revealed right optic atrophy, absent vibratory sense, a right supranuclear facial paralysis, horizontal nystagmus and signs of a bilateral lesion of the pyramidal tract. The patient entered the hospital with the complaint of difficulty in walking, diplopia and visual disturbances. In addition to the foregoing signs and symptoms, examination revealed a left papilledema and a poor pupillary reaction to light. The spinal fluid and blood gave a negative Wassermann reaction. Pleocytosis in the spinal fluid with a large number of polymorphonuclears, but no albumin, and large quantities of sugar in the urine were the other noteworthy features. The condition grew worse; paraplegia in flexion developed, but the spinal fluid became entirely normal. A multiple lesion of the cerebrospinal nervous system was diagnosed. Necropsy revealed hydrocephalus, secondary to an encapsulated cyst in the left lobe of the cerebellum which contained a vascular mural tumor (hemangioblastoma) the size of a hazelnut and which obstructed the sylvian aqueduct and the fourth ventricle, and multiple sclerosis.

In the second case that of a man, aged 44, there was vertigo, gastric distress, unsteady gait, diplopia and genito-urinary anomalies. Later bulbar symptoms and an exophthalmos developed, and the patient was unable to walk or to sit up. There was muscular atrophy with fibrillation in the pectorals, calf and abductor muscles of the thigh; exaggerated tendon and diminished skin reflexes, intention tremor, sensory disturbances in the right lower extremity, a mild bilateral papilledema, horizontal nystagmus with a right supranuclear facial paralysis. Necropsy showed a right cerebellopontile tumor (hemangioblastoma) partially adherent to the eighth nerve and to the fibers of the ninth and tenth nerves; the spinal cord showed foci of necrosis in the middorsal segment.

GEORGE B. HASSIN.

**PATHOLOGY OF SCLERODERMA.** G. RAKE, *Bull. Johns Hopkins Hosp.* **48**:212, 1931.

A case of scleroderma associated with Raynaud's disease and dilatation of the esophagus and colon is described in a woman, aged 30 years. Lesions were present in the cervical sympathetic ganglion cells, and the peripheral arteries showed an endarteritis. The relation of the anatomic findings to the clinical symptoms and signs is discussed, and the present state of our knowledge on the subject is reviewed.

AUTHOR'S SUMMARY.

**INCREASE IN HEART VOLUME IN MITRAL STENOSIS.** E. F. BLAND, G. M. BALBONI and P. D. WHITE, *J. A. M. A.* **96**:840, 1931.

A young man with mitral disease, under observation for nineteen years, lived a relatively active life up to the time of his sudden death, in spite of an extraordinary amount of cardiac enlargement and an extreme degree of auricular dilatation.

The volume (displacement) of the filled heart was 4,600 cc., which is from six to seven times that of a normal sized heart and establishes a record for future comparison. The literature is notably deficient in measurements of heart volume (displacement), a determination that should be a helpful indication of the degree of responsibility of cardiac dilatation in the production of cardiac enlargement. In the case recorded, cardiac hypertrophy was far less a factor than dilatation in causing the extreme enlargement; the weight was 850 Gm., which has often been exceeded.

The left auricle had a capacity of 1,760 cc., a measurement exceeded by only three other cases in the literature. The right auricle held 650 cc., which appears to be a record capacity.

AUTHORS' SUMMARY.

**THE NUMBER OF OPEN GLOMERULI IN ACUTE MERCURIC CHLORIDE NEPHROSIS.** R. A. MOORE and L. M. HELLMAN, *J. Exper. Med.* **53**:303, 1931.

Acute mercurial nephrosis in the rabbit is not associated with a decrease of glomerular circulation.

AUTHORS' SUMMARY.

**THE STRUCTURE OF YOUNG BLOOD PLATELET THROMBI.** K. APITZ, *Centralbl. f. allg. Path. u. path. Anat.* **50**:9, 1930.

Alcohol from 75 to 98 per cent, was painted on the abdominal veins of rabbits, allowed to act from thirty to sixty seconds and then the veins were excised at intervals of from five to one hundred and twenty minutes. In the early stages of thrombi, platelets were deposited on the wall of the injured vein in string-of-pearl fashion with red blood cells occupying the meshes. Later a contraction took place so the red cells were squeezed out and a highly nuclear platelet mass remained. In serial sections, fibrin was never found between the platelets in the mass, although in three of twenty-one veins fibrin threads were found between the red cells at the periphery of the mass. In another series of experiments injections of novirudin were made into the animals in addition to injuring the veins with alcohol. In the second series no typical collections of platelets occurred at the site of injury, although no changes were noted in the platelets themselves. The conclusion is reached that the plasma ordinarily contributes to the agglutination process of platelets in some way, and this property is temporarily checked by substances which prevent clotting.

GEORGE RUKSTINAT.

**IDIOPATHIC CALCIFICATION OF THE CEREBRAL VESSELS.** T. FAHR, *Centralbl. f. allg. Path. u. path. Anat.* **50**:129, 1930.

Disseminated calcification was observed in the capillaries and precapillaries in the white matter of the brain of a man 55 years old. Grossly, this was

evident as tiny thornlike processes about which there was no degeneration. Microscopically, necrosis was not observed but occasional small hemorrhages were seen. The only other disease found at autopsy was a chronic cholelithiasis and a hypostatic bronchopneumonia. In attempting to explain the condition, Fahr hints that the deceased may have imbibed vigantol-like substances which predisposed to calcification because he was known to partake of a variety of medicines frequently.

GEORGE RUKSTINAT.

PANMYELOPHTHISIS (ATROPHY OF BONE MARROW). B. KARITZKY, *Centralbl. f. allg. Path. u. path. Anat.* **50**:177, 1930.

The patient in whom this illness was studied was a woman 20 years old in whom severe epistaxis, metrorrhagia and melena developed. When first seen her blood picture showed 49 per cent hemoglobin, 2,100,000 red blood cells per cubic millimeter, 2,100 white blood cells per cubic millimeter, 89 per cent lymphocytes and 9 per cent polymorphonuclears. Despite a liver diet, roentgen therapy and three transfusions of blood, her condition became rapidly worse; a month and a half after her entrance to the clinic, the red blood cells were only 505,000, the white blood cells, 1,400, the polymorphonuclears, 36 per cent and the lymphocytes, 61 per cent; death from anemia occurred. At autopsy, hemorrhages were noted in the bowel, especially at the tips of the rugae and about the lymph follicles and in the bone marrow of the femurs. There was a marked hemosiderin pigmentation of the cardiac muscle, spleen, kidney and liver and localized lymphocytic infiltrations in the liver. The author considered the possibility of the transfusions of blood leading to the deposits of hemosiderin, but eventually concluded that they were best explained by the anemia. Although the case reported does not fit in, in entirety, with those reported by Frank, the author thinks that it belongs with "panmyelophthisis" rather than with pernicious anemia.

GEORGE RUKSTINAT.

ABNORMAL ORIGIN OF THE LEFT CORONARY ARTERY FROM THE PULMONARY ARTERY. A. J. SCHOLTE, *Centralbl. f. allg. Path. u. path. Anat.* **50**:185, 1930.

In the body of a 2½ month old girl who died of bronchopneumonia, the left coronary artery originated from the sinus of the left pulmonic leaflet. Associated with it were: a marked fibrous myocarditis of the left ventricle with fresh regions of focal necrosis; fatty changes and calcification of the cardiac muscle, and diffuse endocardial thickening of the left ventricle.

GEORGE RUKSTINAT.

THE PATHOGENESIS OF PERFORATING PURULENT AORTITIS AND ITS SEQUELAE. J. F. BUCHALY, *Centralbl. f. allg. Path. u. path. Anat.* **50**:225, 1930.

Acute nonspecific aortitis can be divided on the basis of pathologico-anatomic changes into verrucose, ulcerating or phlegmonous, and etiologically into the metastatic-embolic types and those occasioned by proximity to an infective focus. The author contributes the following interesting case report. A girl, aged 20, affected with chronic furunculosis, fell from a bicycle and injured her back. The injury was sufficient to produce a locus minoris resistentiae, and a staphylococcal abscess formed behind the abdominal aorta. From this abscess a phlegmonous panaortitis developed, ulceration occurred, and a small amount of bleeding took place into the abscess cavity forming a spurious aneurysm. Either at this time or previous to it a septicopyemia had occurred. Then rupture of the aorta occurred into the false aneurysmal sac extended above the diaphragm, and death resulted from bleeding into the right pleural cavity.

GEORGE RUKSTINAT.

THE PATHOLOGIC ANATOMY OF PARAFFIN SICKNESS. N. M. KOLESNIKOW, Centralbl. f. allg. Path. u. path. Anat. **50**:277, 1931.

The investigations on this illness were carried out on workers in the paraffin factory at Grasený. Clinically, the illness manifests itself as miliary comedones, acne, pustules and papules on the skin. The sites of predilection are the shoulders, upper part of the back, thighs and neck—those parts subject to rubbing. The first illness usually starts from two to eight weeks after beginning the work, but in some persons a rash developed as much as four months after stopping work. In biopsy material it was found that the disease starts as an infection of the hair follicles. The lumen of the follicle becomes widened due to the pressing into it of paraffin products. The hair roots become markedly altered, stain more intensely with basic dyes, become brittle and break into small scales. The walls of the distended follicles become thin and then they lose their connection with the epidermis and lie in the cutis in the form of large thin-walled sacs. The surrounding tissue becomes infiltrated with lymphocytes, histiocytes and foreign body giant cells. In some cases the follicles produce a leukocytic reaction, which in a short time leads to their destruction. Clinically, this is manifested in pustules. The follicles or their remnants after acting as foreign bodies in the tissues may lie a long time without causing any reaction. Due to many agencies, inflammation may set in at any time subsequently. In this way new or delayed eruptions are explained. The remnants of follicles eventually become surrounded by firm connective tissue into which they are absorbed. The end-result of the process is diffuse scar formation similar to scleroderma.

GEORGE RUKSTINAT.

THE EFFECT OF LIVER TREATMENT ON THE ANATOMIC CHANGES IN PERNICIOUS ANEMIA. T. FAHR, Deutsche med. Wchnschr. **57**:8, 1931.

Fahr has found that under the influence of liver treatment in pernicious anemia, the characteristic anatomic changes in the marrow, the heart and the liver disappear. The liver treatment, however, does not seem to have any decisive effect on the changes in the spinal cord that may arise in the course of the disease.

### Pathologic Chemistry and Physics

THE ACTION OF ANIMAL TISSUES ON THE SILICIOUS SPICULES OF A FRESH-WATER SPONGE. RALPH G. MILLS, Am. J. Hyg. **13**:224, 1931.

The spicules of the fresh water sponge, *Spongilla fragilis*, are composed of opal, a form of hydrated silica, which is similar to quartz in its chemical reactions. These spicules, when introduced into the tissues of animals, are slowly but definitely dissolved, proving that silica is soluble in the tissue fluids of animals and presumably of man. This confirms the assumption held by many students of silicosis that particles of silicious material are gradually converted into the colloidal state and, as such, exert a fibroplastic influence on the lungs of miners and other industrial workers exposed to silicious material. Definite fibrosis of the lung of a dog into which the spicules had been introduced suggests that there is a concomitant injury attributable to the disappearance by solution of the silicious elements of the spicules.

AUTHOR'S SUMMARY.

THE VALUE OF BRAIN LIPOIDS AS AN INDEX OF BRAIN DEVELOPMENT. FREDERICK TILNEY and JOSHUA ROSETT, Bull. Neurol. Inst. New York **1**:28, 1931.

A brain has been judged efficient or deficient by different investigators according to its size, weight or anatomic or chemical properties. In the present study, Tilney and Rosett give their preliminary observations of the lipid contents of the brain which are so important in the development of myelin and with it in that

of the brain. By using Baug's micromethod for the determination of the lipoids in the blood they found that different divisions of the cerebral cortex possess different chemical constituents. Their study is based on sixty-eight human brains fixed in formaldehyde. They admit the shortcomings of this method, for fixation in formaldehyde affects the phosphatides, a varying amount of phosphorus being lost from the tissues. Yet they arrived at certain highly instructive conclusions. They found that after two years the values of the lipoids remained fairly constant. In ten cases studied for the relative lipid and water contents in the two halves of the cerebral and cerebellar hemispheres, the average difference was less than 0.1 per cent. Profound systemic changes, as well as defective nutritional states, deficiency of vitamins and narcotics such as ether diminish the lipoids in the brain. These are also slightly diminished in infections (pneumonia, tuberculosis, measles, etc.) and very much diminished in nutritional and metabolic disorders. In diseases of the blood and of the cardiovascular organs and in neoplastic diseases the lipid contents are somewhat increased, with a corresponding decrease in the water content. In lesions of the nerves with pronounced parenchymatous degeneration, the lipid content is, with few exceptions, practically unchanged. In intrinsic diseases of the cerebrum and in general systemic diseases, the content of alcohol-soluble fatty substances in the brain is increased, indicating a pronounced degenerative condition—the presence in the brain of an abnormal fatty compound. Further studies are to deal with "fractional and topographical estimations of brain lipoids in embryonic, fetal and postnatal stages of development." A special contribution will deal with the lipid content of the blood, spinal fluid and central nervous system in patients with multiple sclerosis.

GEORGE B. HASSIN.

THE PLASMA PHOSPHATASE IN DISEASE, PARTICULARLY IN BONE DISEASE.  
H. D. KAY, *J. Biol. Chem.* **89**:249, 1930.

The phosphatase activity of the blood plasma, expressed by its ability to hydrolyse betaglycerophosphate, becomes increased in generalized diseases of the bones, such as osteitis deformans, generalized osteitis fibrosa, osteomalacia and rickets. The values may be increased, in extreme conditions, to more than twenty times those characteristic of normal plasma. The increase is correlated, in a general way, with the severity of the disease.

E. R. MAIN.

THE CALCIUM CONTENT OF STRIATED MUSCLE OF RACHITIC ANIMALS. V. G.  
HAURY, *J. Biol. Chem.* **89**:467, 1930.

The calcium content of the striated muscles of the rachitic rat is 44 per cent lower than that characteristic of the muscles of the normal rat.

E. R. MAIN.

SUGAR CONTENT OF SKIN AND MUSCLE IN DIABETIC AND NON-DIABETIC  
PERSONS. H. C. TRIMBLE and B. W. CAREY, JR., *J. Biol. Chem.* **90**:655,  
1931.

The elevation in the concentration of sugar in the blood which characterizes diabetes is accompanied by a marked increase in the sugar contents of the skin and muscle. The average sugar contents of the skin and muscle of nondiabetic persons are 56 and 28 mg. per hundred cubic centimeters, respectively. In diabetes, these averages may reach 144 and 51 mg.

ARTHUR LOCKE.

THE OXIDATION OF FATTY ACIDS OF THE SERUM IN ANEMIA. G. P. WRIGHT  
and B. ARTHUR, *J. Biol. Chem.* **90**:757, 1931.

The blood serum of rabbits rendered anemic through direct loss of blood has an increased content of unsaturated fatty acids owing to the extension of erythropoietic tissue and the consequent extrusion of marrow fat into the blood.

In the presence of ferricyanide, these unsaturated fatty acids consume oxygen and their presence may lead to errors when the determination of the oxygen content of such blood is attempted by the usual Haldane procedure.

ARTHUR LÖCKE.

PRECIPITATION WITH CEREBROSPINAL FLUIDS. A. M. MALLOY, R. L. KAHN and LUCY WESTALL, *J. Infect. Dis.* **48**:203, 1931.

The Kahn reaction and the colloidal gold and mastic reactions depend on the globulin fraction of the spinal fluid, the albumin fraction giving negative results. The globulin fraction gives more sensitive results than the unfractionated fluid, owing undoubtedly to the removal of the protective action of the albumin. After a Kahn test has been performed with a solution of globulin obtained from spinal fluid and the precipitate removed by centrifugation, the supernatant fluid continues to give colloidal gold and mastic reactions. This indicates that the substance or condition responsible for the Kahn reaction is not the same as that responsible for the colloidal gold and mastic reactions. Heating the globulin fraction at 56 C. for as long as two hours exerts little effect on the three reactions. Heating for twenty-four hours decreases the sensitivity, especially of the Kahn reaction. Heating at 65 C. for thirty minutes reduces the sensitiveness of the Kahn reaction more than that of the other two. Heating at 70 C. for thirty minutes reduces the sensitivity of all three reactions. The experiments with heating also indicate that the conditions governing the Kahn reaction are not identical with those governing the colloidal gold and mastic reactions.

AUTHORS' SUMMARY.

THE BLOOD-PIGMENT IN OBSTRUCTIVE JAUNDICE. W. J. GRIFFITHS and GEOFFREY KAYE, *Brit. J. Exper. Path.* **11**:441, 1930.

In six rabbits, ligation of the common bile duct was followed by early and definite icterus, the serum giving a prompt direct van den Bergh reaction. Directly reacting human serum and the serum of rabbits after ligation of the common bile duct resemble in their properties a solution in serum of the "direct reaction" pigment that we have shown to be present in bile. Indirectly reacting human serum resembles in its properties a suspension in serum of pure bilirubin. We have shown that the "direct reaction" pigment yields a diazoreaction over a wide range of hydrogen ion concentrations, viz., when the hydrogen ion concentration of the mixture after addition of the reagent lies between  $p_H$  1.2 and  $p_H$  5, approximately; on the other hand, the pigment of indirectly reacting serum, like bilirubin, yields no prompt direct reaction until the final hydrogen ion concentration of the mixture is adjusted to about  $p_H$  6 by the addition of alkali to the serum before it is mixed with diazoreagent.

AUTHORS' SUMMARY.

THE SUGAR CONTENT OF PLEURAL AND PERITONEAL EXUDATES. J. HERMS, *Beitr. z. Klin. d. Tuberk.* **75**:748, 1930.

An increase in the total number of cells and in the number of polymorphonuclears in pleural and peritoneal exudates indicates intensified inflammatory processes. The protein content is not a reliable criterion, because an increase may be due either to inflammation or to resorption. The main portion of reducing substances is dextrose; its amount in exudates is independent of the amount of dextrose in the blood; it may be equal to that in the blood or may be reduced to 10 mg. The decrease of dextrose in exudates is caused by fermentative anaerobic glycolysis, by adsorption on proteins and by increase of the amount of fluid. Low dextrose values are found in mixed infections, in exudates in chronic pneumothorax, in exudates that become rapidly purulent and in empyemas. Peritoneal exudates rarely show low dextrose contents.

MAX PINNER.

HEAVY METALS IN GALLSTONES. R. SCHÖNHEIMER and W. HERKEL, *Klin. Wchnschr.* **10**:345, 1931.

Copper, zinc, manganese and iron were demonstrated in appreciable amounts in gallstones. In comparison with the concentrations of copper and zinc in the liver, that of copper in the gallstones is greater, and that of zinc less.

EDWIN F. HIRSCH.

### Microbiology and Parasitology

THE FILTRABLE FORMS OF BACTERIA. P. HADLEY, E. DELVES and J. KLIMEK, *J. Infect. Dis.* **48**:1, 1931.

In this investigation we have, for the first time we believe, generated artificially, and subsequently cultivated in pure lines, the filtrable, virus-like stage of a bacterial species. We produced this form (G type culture) by forcing the dissociative reaction on the mother S or R type culture. We grew this G form in pure cultures as a distinct cyclostage before demonstrating that at least some of the cellular elements contained in it were invariably filtrable through Berkefeld N and W candles, as opposed to the S and R type cells, which are not filtrable under the same conditions. From the filtrates we were successful in recovering the G type culture. We have demonstrated, moreover, that the visible elements of the filtrable phase are very different culturally, morphologically, biochemically, serologically, immunologically and in their relation to the Shiga bacteriophage from the "normal" forms of the Shiga species, but that they may be caused to "revert" to the original cell type, possessing all of the original characteristics, including toxicity and susceptibility to bacteriophagic influence. We have shown that the filtrable bodies experience a phase of existence in which they are not visibly cultivable in or on the usual culture mediums and by the usual methods, and that a special technic, such as that used by Hauduroy, must be employed for bringing them into visible development. We have demonstrated, in addition, the high degree of stability enjoyed, in many cases, both by the G type cultures and by the associated virus-like forms when sealed in ampules and stored for periods exceeding two years; we have come to regard them as the most stable forms of the Shiga species. Besides demonstrating the filtrable stage for the Shiga bacillus, we or other members of our laboratory group have shown the existence of analogous phases of culture development in the ontogeny of ten other bacterial species. Their G forms are equally filtrable. Regarding the nature of the G type cultures and the associated filtrable forms, we have concluded that they represent definite cyclostages in the ontogeny of the Shiga species, and that they constitute, in part, at least, the gonidia and the microgonidia. We regard the G type cultures as the visible stage of culture existence lying between the noncultivable (visibly) virus form and the ordinary culture type (S). Both, therefore, have a definite relation to microbic dissociation and are natural products of the dissociative mechanism. The exact cytologic origin of the G forms and the associated virus bodies is not yet entirely clear. There may be several modes of origin, and these may not be the same in different bacterial species. The only origins of which we can speak with any degree of assurance are the lateral or terminal "buds" formed on the rods of old S cultures and the granular inclusions ("gemmules," "gonidia") produced in, and liberated by, the long rods and the filamentous structures that characterize the R type culture. Among the species that we have studied we regard this fungoid R phase as the reproductively mature culture form. The S, we believe, represents a lower, vegetative stage in the ontogeny of the species, while the G type, with its associated virus-like bodies ("microgonidia"), represents the lowest. The specific function of the S type cells is, therefore, vegetative growth; that of the R type cells, reproduction; that of the G forms, dissemination of the species. Regarding the significance of these new filtrable forms for bacteriology, epidemiology and pathology, although much must be left for future study, we have felt justified in discussing in a tentative manner some of the pos-

sibilities that have occurred to us. At the same time we observe that the filtrable phase of ontogeny is not limited to the intestinal bacteria; and it is possible that the analogous forms of other species may possess a different significance from that suggested by the Shiga bacillus, particularly from the point of view of virulence. Regarding the possible relation of these virus-like bodies to the large group of filtrable viruses, we believe that our results offer no grounds for conclusions. At the same time, they perhaps suggest that it will be of advantage, in the future, to incorporate the point of view of microbic dissociation in the study of so-called virus diseases.

AUTHORS' SUMMARY.

FILARIAL PERIODICITY. F. W. O'CONNOR, Porto Rico J. Pub. Health & Trop. Med. 6:263, 1931.

Histologic studies suggest that the hypothesis of Clayton Lane to the effect that the phenomenon of filarial periodicity is due to a simultaneous cyclical parturition of gravid female filariae is correct. A mechanism by which the microfilariae migrate from the vicinity of the parent worms in the lumina of the lymphatic vessels through the walls of the same lymphatic vessels to the adjacent small blood vessels on their way to the general circulation has been demonstrated. While it is not claimed that this is the usual method of migration, the determined nature of its performance suggests that this might be the case.

AUTHOR'S SUMMARY.

### Immunology

PROTECTION OF THE MONKEY BY ANTIPOLIOMYELITIC SERUM. C. P. RHOADS, J. Exper. Med. 53:115, 123 and 137, 1931.

Experiments are reported on the use of poliomyelitis virus neutralized by specific antiserum as an agent to induce active immunity against the experimental disease in monkeys. The results indicate that protection can be conferred on a certain number of the treated animals. The neutralized material did not give rise to symptoms of the disease in any of the monkeys treated. Active poliomyelitis virus, suitably neutralized by admixture with convalescent serum, was without pathogenic effect when given by repeated nasal instillations.

The experiments described in this paper raise the question whether, therapeutically considered, the antipoliomyelitic horse serum should be regarded as an exact equivalent of, and hence employed as a perfect substitute for, convalescent serum. This question can only be answered by further experiment and observation.

A comparison has been made of the neutralizing value of pooled convalescent monkey serum for the filtered virus of poliomyelitis before and after a series of reinforcement injections of the same virus strain. The strength of the pooled convalescent serum is increased by the reinforcing procedure. The original monkey convalescent serum had a neutralization value much below that of a pooled human convalescent serum. By reinforcement, the neutralization value of the monkey serum was brought approximately to that of the human serum. One sample of serum from a supposedly normal child of 8 years exhibited a neutralizing value approximately equal to that of a pooled human convalescent serum and the reinforced pooled monkey serum.

AUTHOR'S SUMMARIES.

MOUSE PROTECTION TESTS FOR ANTIBODY IN PNEUMOCOCCUS PNEUMONIA. F. T. LORD and E. L. PERSON, J. Exper. Med. 53:151, 1931.

Though in general in pneumococcus pneumonia the appearance of protective substance coincides rather sharply with the fall in the temperature, antibodies may appear spontaneously in the blood serum as early as the third or fourth day, and crisis and recovery may be delayed until between the sixth and the tenth day.



Recovery at times occurs without demonstrable protective substance in the blood of patients in whom protection later develops. The amount of antibody developed in the course of pneumococcus pneumonia is small, and in the majority of the patients tested was insufficient to protect against more than 100 lethal doses of homologous pneumococci and never against more than 10,000 lethal doses. Treatment with Felton's antibody late in the course of the disease materially increases the amount of protective substances in the blood. In fatal cases a high degree of protection may be established by treatment. After the third day, doses of more than 200,000 Felton units are usually necessary to produce a greater degree of protection than might otherwise be expected. The formation of protective substances by the patient himself is not an assurance against the progress of the infection to a fatal termination. Protective substance in the blood and pneumococcic septicemia may occur simultaneously.

AUTHORS' SUMMARY.

IMMUNIZATION AGAINST VACCINIA BY NON-INFECTIVE MIXTURES OF VIRUS AND IMMUNE SERUM. C. P. RHOADS, J. Exper. Med. **53**:185, 1931.

Vaccine virus and specific immune serum mixed in a proportion that will not produce a lesion when inoculated intradermally will induce immunity when instilled into the nasal cavities of rabbits. The mixture is also effective when inoculated subcutaneously. Immunity is brought about with a minimum systemic and no local reaction. When the mixtures are incompletely neutralized, generalized vaccinia results.

AUTHOR'S SUMMARY.

THE PULMONARY PERMEABILITY IN DOGS IN RELATION TO ANAPHYLACTIC SHOCK. N. P. SHERWOOD and O. O. STOLAND, J. Immunol. **20**:101, 1931.

Our results indicate that in heart-lung perfusion experiments on normal and sensitized dogs, the phenomena consisting of a reduction in rate of flow of perfusion fluid of from 50 to 75 per cent, the development of a rubbery consistency of the lungs and the appearance of a tracheal exudate, all in from four to seven minutes, are not peculiar to the sensitized dog as judged by the blood pressure and coagulation time phenomena. While these do not correlate with other and at present accepted criteria of sensitization, yet a quantitative study of our data suggests that a probable change in permeability in heart-lung tissue, perhaps independent of anaphylaxis, does frequently result from the injection of horse serum in a dog. Experiments in passive sensitization show that marked variation exists in either ability to take up antibody or refractoriness to the shocking dose of antigen. This observation indicates that negative results on a single recipient are not an accurate criterion of deficiency or absence of antibodies in the donor's blood.

AUTHORS' SUMMARY.

ISOHEMAGGLUTINATION: THE WORK OF JAN JANSKÝ WITH A CRITICAL ANALYSIS. J. A. KENNEDY, J. Immunol. **20**:117, 1931.

This article reviews the original publication by Janský on isohemagglutination in *Archives Bohèmes de médecine clinique* (8:85, 1907).

THE ANTIGENIC PROPERTIES OF BACTERIOPHAGE. E. W. SCHULTZ, J. S. QUIGLEY and E. L. WOOLSEY, J. Immunol. **20**:149, 1931.

Judged by plaque counts, the neutralization of phage by antiphagic serum was found to follow quite closely the curve of the velocity of an adsorption reaction. No evidence was obtained to indicate that the reaction follows a logarithmic curve. Results were obtained which indicate that in the neutralization the reaction under set conditions takes place in definite proportions, provided the reaction is allowed to go to completion. The Danysz-Bordet effect was elicited, when phage was

added in small increments to serum and, similarly, when serum was added to phage in small increments. Neutral serum-phage mixtures could not be dissociated by dilution with physiologic solution of sodium chloride.

FROM AUTHORS' SUMMARY.

SKIN REACTIONS TO POLLEN EXTRACTS IN RABBITS. H. W. CROMWELL and M. B. MOORE, *J. Immunol.* **20**:161, 1931.

Skin sensitivity to pollen extracts has been demonstrated in actively immunized rabbits, trypan blue being used as an aid, after the method of Ramsdell (*J. Immunol.* **15**:305, 1928, and **16**:509, 1929). The reaction is specific, and is more delicate than the precipitin test as indication of an immune response of the animal. The localization of the trypan blue also occurs in the abdominal fluid of the immune animal following intraperitoneal injection of the pollen antigen. Passive transfer of the skin sensitivity from immune to normal rabbit has been accomplished by the use of large quantities of immune serum.

AUTHORS' SUMMARY.

SKIN REACTION TO AGAR AFTER INJECTION OF MENINGOCOCCUS FILTRATE. G. M. SICKLES, *J. Immunol.* **20**:169, 1931.

Intravenous injections of agar may be followed by reactions in skin areas prepared by injections of meningococcus toxic filtrate. Local reactions did not occur at the site of intracutaneous injections of agar when followed by intravenous injections of agar or of toxin. No reactions were obtained after the injection of other nonbacterial substances such as galactose, gelatin, serum and india ink, intravenously, following intracutaneous injection of meningococcus toxic filtrate.

AUTHOR'S SUMMARY.

PROMOTION OF PHAGOCYTOSIS BY VARIOUS CHEMICAL SUBSTANCES. RUTH TUNNICLIFF, *J. Infect. Dis.* **48**:161, 1931.

Rabbits receiving intravenous injections of solutions of calcium gluconate, calcium chloride, sodium salicylate, sodium iodide, dextrose and neoarsphenamine showed a marked increase in phagocytosis by leukocytes in the blood. Physiologic solution of sodium chloride, distilled water, milk, sodium phosphate and mercurochrome-220 soluble in water or in dextrose injected intravenously into rabbits appeared to cause no increase in phagocytosis. Intravenous and intramuscular injections of calcium gluconate produced the same high degree of phagocytosis in rabbits, intravenous injections acting twenty-four hours earlier than intramuscular injections. A high degree of phagocytic activity was obtained in a man given intravenous injections of calcium gluconate, followed by intramuscular injections and finally by the use of the drug by mouth. With the discontinuation of the drug, phagocytosis returned to normal. In vitro, dilute solutions of calcium gluconate, sodium iodide, dextrose and mercurochrome in dextrose stimulated phagocytosis, while solutions of sodium phosphate, milk and mercurochrome in water did not appear to increase phagocytic activity.

AUTHOR'S SUMMARY.

CUTANEOUS HYPERSENSITIVENESS IN GUINEA-PIGS INFECTED WITH BRUCELLA ABORTUS. A. C. S. STROEM, *J. Infect. Dis.* **48**:167, 1931.

Guinea-pigs infected with *Brucella* showed cutaneous hypersensitiveness to abortion even when gross anatomic lesions were absent; those infected with one strain of *Brucella* showed cutaneous hypersensitiveness to abortin prepared from five other strains. Guinea-pigs inoculated with heat-killed vaccine or with a mixture of vaccine and kieselguhr did not show cutaneous hypersensitiveness to abortin. Guinea-pigs infected with *B. abortus* gave negative reactions with tuberculin. Tuberculous guinea-pigs gave slight, somewhat atypical reactions with abortin, presumably due to increased nonspecific hypersensitiveness.

AUTHOR'S SUMMARY.

LOCAL SKIN REACTIVITY TO STREPTOCOCCUS HEMOLYTICUS-SCARLATINAE. G. SHWARTZMAN, J. Infect. Dis. **48**:183, 1931.

The skin reactivity was induced by intradermal injection of a potent filtrate of *B. typhosus* "agar washings." The streptococcal reacting factors were present in live cultures of the streptococcus in fluid medium. They were also present, though in lesser concentration, in filtrates of cultures, in toluene-killed cultures and in the supernatant fluid of centrifugated cultures of *S. hemolyticus-scarlatinae*. Reacting factors in stable form were obtained by alcohol precipitation of live cultures. The reacting factors persisted in the material after the removal of bacterial bodies and could be reprecipitated with acid alcohol.

FROM AUTHOR'S SUMMARY.

"ZONING" PHENOMENON IN COMPLEMENT FIXATION. B. S. LEVINE, J. Infect. Dis. **48**:189, 1931.

The results, omitting mathematical formulas, are as follows: In the serum of most adults there are various types of immune substances. On the addition of cholesterized alcoholic beef heart suspension, the action of which is considered nonspecific, interfaces are created at the suspended particles of the colloid. The so-called immune complexes concentrate at such interfaces in spherical and spheroid configuration. With a decrease in the dilution of the serum, the dispersion of the suspended complexes increases, leading to an increase in the surface area of the sensitized spheres in accordance with a special mathematical formula. With a ratio of the dispersion in the serum of the higher dilution to that of the lower exceeding 8:1, the consumption of complement by volume  $v/2$  of full strength serum will be greater than the consumption of complement by volume " $v$ " of full strength serum when both are diluted to the same final volume with saline and the antigen suspension. Hence, "zoning" will appear in all serums in which the consumption of complement by the so-called "nonspecific" antibodies in the first tube of the cold fixation procedure just exceeds, equals or nearly equals one unit.

EDNA DELVES.

DETOXIFYING AND DISINFECTING PROPERTIES OF SODIUM SALICYLATE. K. E. BIRKHAUG, J. Infect. Dis. **48**:212, 1931.

Saturated solution of sodium salicylate neutralizes diphtheria and tetanus toxins in vitro without destroying their antitoxinogenic capacity. Neutralization proceeds in direct proportion to the concentration of sodium salicylate, the duration of contact and the degree of temperature. Simultaneous injection of pure toxin and the amount of sodium salicylate that produces a neutral mixture in vitro fails to avert death from diphtherial toxemia, although the period of survival is prolonged slightly. No neutralizing effect by the saturated solution of sodium salicylate on the toxic filtrates of hemolytic and nonhemolytic was found. This separates these "toxins" from the true exotoxins of diphtheria and tetanus. The bactericidal action of sodium salicylate on the common pathogenic bacteria is about one-tenth that of phenol. The precise mode of action of sodium salicylate in vivo remains obscure. Its dual capacity to exercise antitoxic and antiseptic action in vitro suggests the hypothetic possibility that its therapeutic success in certain infectious diseases is effected by weakening the pathogenic micro-organisms and their capacity to secrete the deleterious exotoxins.

AUTHOR'S SUMMARY.

TUBERCULIN SENSITIVITY OF GUINEA-PIGS UNDER ACIDOTIC DIET. E. H. MICHALOWSKY, Ztschr. f. Tuberk. **59**:321, 1931.

Tuberculous guinea-pigs whose alkali reserve was markedly decreased by an acidotic diet showed weaker skin reactions to tuberculin than control animals under a normal diet and with a normal alkali reserve.

MAX PINNER.

## Technical

INOCULATION OF TUBERCULOUS MATERIAL INTO THE LYMPHATIC GANGLIONS.  
G. NINNI, *Ann. de l'Inst. Pasteur* **45**:433, 1930.

Rapid diagnosis is secured, particularly with injections of pleural or cerebro-spinal fluids, with numerous organisms present in the cervical lymphatic ganglions of the guinea-pig. Results are secured within from eight to twelve days after the injection of small doses. Selective development of the tubercle bacillus, with destruction of other types of organisms that might be present, seems to occur.

M. S. MARSHALL.

COMPLEMENT FIXATION AND THE SERUM LABILITY TEST IN TUBERCULOSIS.  
G. IVANOVICS, *Beitr. z. Klin. d. Tuberk.* **75**:657, 1930.

Daranyi's lability test and the determination of the refraction of the serum aid considerably in the diagnosis and prognosis in tuberculosis. They yield constantly positive results in exudative tuberculosis, while they are positive in about two thirds of the fibrotic cases. Complement fixation yields only from 50 to 70 per cent positive results, and it is not strictly specific.

MAX PINNER.

BACTERIOLOGIC DIAGNOSIS OF TUBERCULOSIS. J. MOURIZ, *Klin. Wchnschr.* **9**:2249, 1930.

The Hohn method is the best for isolating tubercle bacilli from contaminated material. The concentration of sulphuric acid in the technic described by Löwenstein should be reduced to a minimum, but not below from 4 to 5 per cent. The potato-egg medium used afforded an abundant and rapid growth of tubercle bacilli. In order to maintain adequate moisture, a specially constructed tube is recommended.

AUTHOR'S SUMMARY.

THE USE OF ILLUMINATING GAS IN THE PREPARATION OF MUSEUM SPECIMENS.  
A. SCHULTZ, *Klin. Wchnschr.* **10**:213, 1931.

The use of Jore's solution saturated with carbon monoxide has been found better for fixing and preserving the color of museum specimens than the previously suggested Kaiserling iodine solution saturated with carbon monoxide.

EDWIN F. HIRSCH.

PREPARATION OF PURE AND STABLE PLASMA FROM MAMMALIAN BLOOD BY CENTRIFUGATION. H. J. FUCHS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **69**:305, 1930.

The blood is drawn under special precautions to prevent damage to the platelets and is centrifugated immediately at 14,000 revolutions a minute. The centrifuge is described in *Zeitschrift für Immunitätsforschung und Experimentelle Therapie* (**69**:180, 1930). The secret of success in obtaining stable plasma lies in the complete sedimentation of the platelets before they can disintegrate. Plasma has been obtained by Dilezenne and others according to this principle from birds, reptiles and fishes, with much greater ease because their platelets are not so fragile as the mammalian ones. Fuchs concludes from his work that native plasma does not contain any free prothrombin.

# Society Transactions

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## AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

*Annual Meeting, McGill University, Montreal, April 9, 10 and 11, 1931*

SAMUEL R. HAYTHORN, *Vice-President, Presiding*

C. PHILLIP MILLER, JR., *Secretary*

CHARACTER OF THE EXCITATION WAVE IN AURICULAR MUSCLE. FRANK N. WILSON, A. GARRARD MACLEOD (by invitation) and PAUL S. BARKER (by invitation), University of Michigan.

When one electrode of a sensitive galvanometer is placed on the auricular surface and the other on one of the extremities, the resulting curve may be considered a record of the potential variations of the cardiac electrode alone.

The curves obtained from certain auricular regions by this method are, so far as their general outline is concerned, of relatively simple form. The shape of these curves indicates that the electrical disturbances associated with the excitatory process may be represented by a positive pole, or source, just ahead of the crest of the excitation wave, and a negative pole, or sink, just behind it. The approximate interval between the sink and the source may be determined from the experimental curve.

When both electrodes are placed on the heart, one on an injured and the other on an uninjured region, the resulting curve, so far as it is monophasic, is due to potential variations within the injured region.

A theoretical investigation of the electric field surrounding a polarized membrane shows that these observations are in accord with the so-called membrane theory.

NUTRITIONAL ENCEPHALOMALACIA IN CHICKS. ALWIN M. PAPPENHEIMER and MARIANNE GOETTSCH, Columbia University, and the Storrs Experiment Station, Storrs, Conn.

A severe disease of the central nervous system develops in chicks maintained on a diet of milk powder, casein, lard, starch, yeast, salt mixture, cod liver oil and filter paper. Growth is normal until the appearance of symptoms (usually between the eighteenth and the twenty-fifth days). These consist of ataxia, tremors, retraction or twisting of the head, clonic spasms of the legs and stupor. There is found extensive ischemic necrosis of the brain, with hemorrhages and capillary thromboses. The lesions are usually in the cerebellum, less frequently in the cerebrum and rarely in the medulla.

Protective experiments have been carried out with whole wheat, wheat bran, wheat germ, white flour, wheat gluten, wheat ash, wheat germ oil, yellow corn, dried alfalfa, meat scraps, yeast, ether extract of whole wheat, extracted residue, spinach, cabbage and lettuce. Thus far, complete protection has been obtained with none of these substances, but the introduction of whole wheat, spinach and lettuce appears to reduce the incidence of the disease at the levels fed to approximately 20 per cent, as compared with 60 per cent in the untreated controls on diet 108. Toxicity of the cod liver oil can be excluded as a factor. Irradiation of the chicks with the quartz-mercury lamp does not protect. A natural diet composed of a mixture of grains gives protection; this is not affected by autoclaving.

**HYPERPLASIA OF THE SKIN OF THE RAT AND MOUSE FROM SULPHYDRYL.**  
STANLEY P. REIMANN, The Lankenau Hospital Research Institute, Philadelphia.

The sulphydryl group ( $-SH$ ) is the naturally occurring stimulus to cell division. Sulphydryl compounds stimulate mitosis not only in a number of different living forms, but also in a variety of different expressions of cell division such as normal growth, regeneration, repair of wounds, etc. The details and a theory of chemical regulation of growth can be found in the publications of Hammett (*Protoplasma* 11:382, 1930).

To discover what happens when the sulphydryl group is offered to normal cells, as of the skin, a series of rats and mice was painted with thiocresol in alcoholic solution, in lanolin, in petrolatum and in other vehicles. To the same animals on another part of the body was applied, as control, a corresponding concentration of cresol in the same vehicles.

In about 20 per cent of the animals, when the material was applied in a greasy vehicle, an itching rash developed after about two weeks of three applications weekly. This gradually disappeared over a period of another two weeks, and at the end of six weeks both the animals without the rash and those that had developed it presented the same end-results, namely, a skin obviously thickened and denser to the touch. Histologic examination showed this thickening to be due to a proliferation of epithelial cells until the normal two to four layers of cells in the skin of the mouse and rat became transformed to eight or ten or more layers. The structures preserved their normal relations except for this higher differentiation. The connective tissue beneath also reacted by the production of more cells and greater vascularity. There was, however, no inflammatory exudation in any of the animals. Pathologically, it presented the picture of pure hyperplasia.

In this material, Hammett is studying the type of proliferation, the differentiation of the cells, the differential reactions between the epithelial and the underlying connective tissue, etc., and is preparing reports of his studies for publication.

**VARIATIONS IN THE TEMPERATURE OF HEALTHY AND TUBERCULOUS BIRDS.**  
DAVID L. BELDING, Boston University School of Medicine and Evans Memorial.

The study of diseases of birds and the use of birds as experimental animals require a knowledge of the variations in the temperature of normal birds. My observations were made on pheasants, mallard ducks and chickens, and are concerned with the physiologic and pathologic factors that influence the temperature of the body.

The temperature of the body varies with both the species and the individual bird. It increases with age and is slightly higher in the female than in the male. Extreme care is necessary to reduce errors due to the methods of taking the temperature and of handling the birds. Excitement with or without demonstrable muscular activity definitely increases the temperature. This fact throws doubt on the reliability of most of the published records of the temperature of birds.

Birds show a daily rhythm of temperature, the maximum temperature occurring at midday and the minimum at midnight. Exercise produces an initial rise followed by a fall to a subnormal degree, varying in rapidity with species, age, sex and type of exercise. Fatigue and starvation produce a subnormal temperature. Tuberculous birds have a slightly lower temperature than healthy birds.

**THE CELLULAR REACTION TO INFECTION WITH TUBERCLE BACILLI; EXPERIMENTS ON THE TESTES OF NORMAL AND IMMUNIZED GUINEA-PIGS.**  
ESMOND R. LONG, ARTHUR J. VORWALD and LILLIAN DONALDSON (all by invitation), the University of Chicago.

Virulent tubercle bacilli of the human type in a dosage of 0.1 mg. per kilogram of weight of the animal were injected into the testes of (group A) normal

guinea-pigs, the testes of (group B) tuberculous guinea-pigs, the locally immunized testes of (group C) guinea-pigs that had received from one to two months previously four intratesticular injections, at weekly intervals, of killed tubercle bacilli, and the previously untreated testis of (group D) guinea-pigs that had received injections of dead tubercle bacilli in one testis. Guinea-pigs of each series were killed after periods of one, three, six, ten, sixteen, twenty-four, forty-eight and seventy-two hours. In each series the great importance of the polymorphonuclear leukocyte was shown. It was the predominant cell in the middle stages of the early reaction in every case, and was highly active in two respects: (1) phagocytosing tubercle bacilli and (2) localizing them by concentration in deep regions of the organ. The speed and intensity of the cellular reaction increased in the different series as follows: (1) the testis of the normal animal, (2) the testis of the tuberculous animal, (3) the nonimmunized testis of the animal with local immunization of the opposite testis and (4) the testis of the animal with local immunization of both with testes. A striking feature was the intense response of polymorphonuclear leukocytes in the locally immunized testis in spite of the fact that large mononuclear leukocytes (monocytes, clasmatocytes, etc.) were already present in large numbers as a result of the immunizing process. In the tuberculous animals exudation of plasma and tubular degeneration were much more intense than in animals of the other series (tuberculin reaction). In each series after from twenty-four to seventy-two hours large mononuclear cells phagocytosed the polymorphonuclear cells containing bacilli, so that after three days the reaction was of the familiar early epithelioid type, the bacilli being seen almost exclusively in large mononuclear exudate cells.

EXPERIMENTAL INFECTION WITH TUBERCULOSIS. ROBERT G. BLOCH (introduced by Russell M. Wilder), the University of Chicago.

An emulsion of virulent tubercle bacilli in saline solution injected intratracheally quickly produces widespread pulmonary tuberculosis, even when very small numbers are injected. It is therefore impossible to produce by this method isolated primary tuberculous infects and complexes. A method was developed to inject intratracheally very small amounts of iodized poppy seed oil 40 per cent loaded with virulent tubercle bacilli and to demonstrate the focus of the iodized oil in the lung by x-ray pictures. On killing the animals after from three to four weeks, the primary tubercle is found where the oil droplet was demonstrated. Comparative microscopic studies indicate that lesions produced in the described way do not differ essentially from the ordinary ones. If they are permitted to develop into more advanced stages, it is found that the oil slightly delays their progress.

CYTOLOGIC REACTIONS IN EXPERIMENTAL MONILIAL ABSCESES. GEORGE H. ROBINSON and SAMUEL R. HAYTHORN, Singer Research Laboratory, Pittsburgh.

A strain of a pathogenic monilia was used to produce lesions that afforded a study of an exudate containing numerous cells closely resembling the Dorothy Reed cells of Hodgkin's disease.

The lesions produced by the monilia began as small abscesses, which persisted for several days and healed with an unusual type of granulation tissue in which blood vessels were rarely found and in which two strains of mononuclear cells were prominent. One was typical of the ordinary mononuclear phagocytes, and these fused to form foreign body giant cells about moniliae and fibrin. The other type was a large cell with a cartwheel-like nucleus and prominent nucleoli, which were often multiple and which had a slightly granular basophil protoplasm. These cells were found in the walls of the abscess, in the newly formed reticulum and in the lymphoid tissue in nearby lymph nodes, where they were sometimes found in mitosis. The large cells were studied for means of identification in relation to cells of the types constituting reticulo-endothelium, lymphoblastic tissue, early

cells of the bone marrow, or cells of the megakaryocyte series. They were not identified, but their close resemblance to the large cells typical of Hodgkin's disease indicated the danger of diagnosing that disease on the presence of these cells alone.

GROWTH REQUIREMENTS OF GONOCOCCUS IN A LIQUID NONPROTEIN MEDIUM.

ALDEN K. BOOR (by invitation) and C. PHILLIP MILLER, JR., the University of Chicago.

In an effort to adapt their protein hydrolysate medium (*Proc. Soc. Exper. Biol. & Med.* **28**:370, 1931) for use in liquid cultures of large volume, the authors encountered certain difficulties that led to the present study. The medium consists essentially of an acid hydrolysate of powdered egg-white, which has been cleared with aluminum hydroxide cream, adjusted to the proper hydrogen ion concentration and buffered, and to which 1 per cent dextrose has been added. It has the advantage of being free from antigenic proteins.

In order to study the effect of varying oxygen tensions, large tubes containing 10 cc. of medium were inoculated with approximately equal numbers of organisms, and the air replaced by mixtures of oxygen, nitrogen and carbon dioxide in known concentration, the percentage of oxygen varying from 10 to 100. The cultures were allowed to stand undisturbed in the incubator. Growth was slow and not very abundant, but it occurred in all tubes and was best in those containing 40 and 60 per cent oxygen, respectively. Varying the carbon dioxide tension seemed to have no influence on the growth when the hydrogen ion concentration was kept constant.

When atmospheric air was bubbled through the inoculated medium, growth was much accelerated, so that at the end of fifteen hours the yield was more abundant than it had been in any of the undisturbed tubes after six days. Agitating a flask of the medium by double eccentric rotation (so that the sides of the flask were being alternately wetted and exposed to the air) resulted in as good growth as was obtained by aeration.

EFFECT OF FILTRATION AND OF VARIOUS CONSTITUENTS OF TISSUE ON THE VIRUS OF POLIOMYELITIS. CLAUS W. JUNGEBLUT, Columbia University.

Titration of suspensions of virus-infected cord of varying percentages permits the finding of a particular concentration with maximum virulence, the concentrations below and above which show progressively diminished virulence.

Berkefeld-N filtrates of suspensions of virus-infected cord are frequently more virulent than the corresponding unfiltered suspensions, suggesting removal of an inhibitory factor from the emulsion.

Spinal cord from a normal monkey added in vitro to filtrates of virus has no appreciable effect on the virulence of the virus. Spinal cord from a convalescent monkey, in one instance, effected a complete inactivation of the infectious agent.

Testicle from a normal monkey added to filtrates of virus in vitro appears to adsorb the greater part of the virus, so that practically all the virus is found in the sediment and very little or none in the supernatant fluid. The testicles of monkeys do not all display this adsorptive effect to the same extent.

Testicle from a normal rabbit is incapable of adsorbing the virus in vitro, but rather tends to enhance the infection.

THE MECHANISM OF THE ACTION OF BACTERIOTROPIN: THE RELATIVE DEGREE OF PHAGOCYTOSIS BY POLYMORPHONUCLEAR LEUKOCYTES AND BY MONOCYTES. BALDUIN LUCKÉ, MAX STRUMIA (by invitation), STUART MUDD and MORTON McCUTCHEON, the University of Pennsylvania.

In studies on the mechanism of the phagocytosis-promoting action of normal and of immune serums and of their protein fractions, the polymorphonuclear leukocyte has generally been used as the phagocytic cell. The influence on the other principal type of phagocyte, the large monocyte, has received little attention.



The present experiments were designed to compare, quantitatively, the effects of normal and of immune bacteriotropins on the phagocytic behavior of the polymorphonuclear leukocyte and the large monocyte. The relative degree of phagocytosis by these cells was determined toward various kinds of particles (collodion, different strains of acid-fast bacteria, pneumococci, pyogenic cocci, yeasts, etc.).

It was found that:

1. In salt solution alone certain particles are taken up more readily by monocytes than by leukocytes (mammalian tubercle bacilli, collodion particles, etc.).
2. Except under special conditions, deposits of normal or of immune serums on particles causes a parallel increase in phagocytosis by both monocytes and polymorphonuclear leukocytes.
3. The degree of phagocytosis in response to bacteriotropins is, in general, more pronounced with the polymorphonuclear leukocytes than with the monocytes.

IMMUNITY OF TISSUES: CONDITIONS INFLUENCING THE FIXATION AND DESTRUCTION OF BACTERIA IN THE TISSUES. PAUL R. CANNON, E. F. NECKERMANN (by invitation) and F. L. SULLIVAN (by invitation), the University of Chicago.

When approximately equal quantities of bacteria are injected simultaneously into normal rabbits and into others previously immunized, the bacteria steadily leave the blood stream, but at an accelerated rate in the immunized animals. Quantitative studies of organs such as the spleen, liver, bone marrow and lungs indicate that the number of colonies developing per unit volume of tissue is definitely greater, during the first few minutes after injection, in the immunized than in the normal animals. After from thirty minutes to forty-five minutes, however, more colonies per unit volume are present in the tissues of the normal animals.

Histologic studies show that immunization leads to hyperplasia of the splenic follicles as well as of the red pulp, with an increased intensity of phagocytosis in the latter. Furthermore, staphylococci ingested by phagocytes in the red pulp of the immunized animals seem to become swollen and disintegrated more extensively and more rapidly than in the normal control animals. The conclusions are that immunization, by its activation of the mesenchymal tissues, especially in the spleen and liver, elevates the capacity of the tissues to remove bacteria from the blood stream and increases the effectiveness of such tissues in the destruction of the bacteria removed.

THE DEVELOPMENT OF CARCINOMA FROM POLYPI OF THE COLON. H. E. ROBERTSON, the Mayo Clinic, Rochester, Minn.

Studies of the polypi of the colon in various stages of development help to formulate the pattern of their growth and of the stages of transition between them and cancer.

THE PATHOLOGY OF PICK'S DISEASE. CLAUDE S. BECK, Western Reserve University.

The condition designated as "pericarditic pseudocirrhosis" in 1896 and subsequently known as Pick's disease consists of chronic pericarditis as the primary factor and enlargement of the liver, ascites and other signs of circulatory failure as secondary factors. Tuberculosis is generally regarded as the common etiologic factor of the pericarditis, but other forms of infection play a more important rôle. The acute infection usually subsides, leaving only scar formation or deposition of calcium in the pericardium.

The Pick syndrome was produced experimentally by the application of Dakin's solution in the pericardial cavity. The pericardium became scarified. Adhesions between the heart and the pericardium were not necessary to produce the condi-

tion. The essential factor, as shown by roentgenograms, was the contracture of the scar. This impaired the filling of the heart; the venous pressure rose and the minute volume output of the heart decreased. Ascites and enlargement of the liver developed. The surface of the liver showed a deposition of fibrin and fibrous tissue, and this finding indicated that the classic "Zuckergussleber" was not necessarily due to infection. Hydrothorax and subcutaneous edema developed late and were terminal manifestations. The electrocardiograms showed slurring and decreased voltage.

The method provides material for investigation by both clinician and surgeon.

A SPECTROPHOTOMETRIC STUDY OF BLOOD SOLUTIONS. R. P. KENNEDY, the University of Rochester.

Some observations on solutions of blood that correlated values for oxygen capacity with quantitative measurements of color were published in an earlier paper (*Am. J. Physiol.* **79**:346, 1927). Certain optical constants were also evaluated for oxyhemoglobin. The data included here represent a similar spectrophotometric study of blood pigment in its reduced form hemoglobin. Spectrophotometric data of this kind have been available in the literature for a long time, but these were derived from the measurement of the color of blood reduced by chemical means or reduced by allowing it to putrefy (Hari, P.: *Biochem. Ztschr.* **115**:54, 1921). It seems desirable, therefore, to gather additional information concerning the change in the color of solutions of blood when they are simply subjected to reduced oxygen pressure. Also, it is important to ascertain whether or not the color returns to the original in the reverse reaction. In this work the change in color from the oxidized to the reduced form was studied in three steps: First, a spectrophotometric curve was plotted for a solution of oxyhemoglobin. Second, the oxygen was removed by diminished pressure and the corresponding color recorded. Third, the same solution was resaturated with air and a second oxyhemoglobin curve made and compared with the first. Thus, the reversible reaction of oxygen and hemoglobin may be studied from the standpoint of colorimetry.

In addition to the examination of the color of dilute solutions of blood, extracts were made of skeletal muscle of perfused dogs and similar spectrophotometric measurements made on these extracts in both the oxidized and the reduced forms. It is interesting to compare the shape of spectrophotometric curves of blood pigment with that of such curves obtained from blood-free skeletal muscle. Only slight differences are observed, and these are discussed. A comparison of the curves of oxidized and reduced pigment opens up the interesting possibility of determining an oxygen dissociation curve for hemoglobin in dilute solutions.

THE SIGNIFICANCE OF WATER BALANCE IN EPILEPSY. F. B. BYROM (introduced by Russell M. Wilder), the University of Chicago.

The water balance in epileptic patients was determined by the method of Newburgh.

The ketogenic diet causes an immediate loss of body water, equilibrium then being established at a lower level. Two factors are mainly responsible: First, the removal of protein-sparing carbohydrate from the diet causes a temporarily negative nitrogen balance and a proportionate liberation of water held in physical combination. Second, a loss of fixed base (sodium) occurs, which is also arrested after a few days by mobilization of the urea-ammonia mechanism. The individual convulsions also cause a loss of water. Further attacks may, however, occur during this negative phase, while the reactionary phase of water retention that follows may be free from attacks. It is concluded that the convulsions in epilepsy cannot be explained solely on the basis of disturbed water balance.

## RELATION OF SULPHUR TO EXPERIMENTAL ANEMIA. O. M. GRUHZIT, Parke, Davis &amp; Company, Detroit.

A severe hemolytic anemia was induced in dogs by feeding onions, onion fractions and some disulphide derivatives of the general formula R-S-S-R, which are chemically closely related to oil of onions, the allyl disulphide. The work on disulphides was followed by a similar study of monosulphides of the general formula R-SH. The radical "R" in these compounds represents an aliphatic or aromatic group. This study covered propyl, tolyl, benzyl, diphenyl disulphides, l-cystine and dithioglycollic acid. In the series of monosulphides were included thiophenol, thiocresol and butylisothiurea. Since some of these compounds are sulphur homologues of phenol, the latter was similarly studied.

In these series feeding of the benzyl disulphide, cystine, dithioglycollic acid, butylisothiurea and phenol caused no anemia in dogs. The feeding of propyl, tolyl, diphenyl disulphides and the monosulphides, thiophenol and thiocresol, caused severe acute hemolytic anemia. The maximum hemolytic effect was reached in from five to seven days. The hemoglobin dropped to less than 20 per cent by Sahli, the number of the red cells decreased to less than 1,000,000 per cubic millimeter. The white cell count rose to 60,000 per cubic millimeter. The reticulocytes at times constituted 85 per cent of the total red cells. The red cells showed various degenerative forms, and microscopically it was difficult to differentiate the blood picture from that of pernicious anemia. The color index also approached 1 and at times it was over 1. The leukocyte and reticulocyte counts always remained high and increased in proportion to the severity of the anemia. At no time was I able to induce aplasia of the bone marrow. In some of the dogs the hemoglobin has been kept at a level around 20 per cent (by Sahli) for the last six months without difficulty, and no tolerance has developed, except to diphenyl disulphide and probably to the monosulphides tested. Discontinuation of the feeding of sulphide reverted the condition of the blood to normal.

The anemia caused by the feeding of sulphide is probably of the same general type as that induced by compounds of the type of phenyl hydrazine. The hemolytic action of compounds of the type of sulphide is probably related to a fixed stability of sulphur to the radical group. In benzyl disulphide the linkage of the sulphur to the radical is unstable, the compound undergoes rapid degeneration, and no anemia was induced by this compound. The tolyl disulphide, an isomer of benzyl disulphide with sulphur in stable relation to the radical, possessed high hemolytic action. The thiophenol, the sulphur homologue of phenol, possessed extremely high hemolytic activity, yet phenol had no hemolytic action. In cystine, thioglycollic acid and butylisothiurea the sulphur is bound weakly to the radical. These compounds were devoid of hemolytic properties.

In undertaking this study I was interested to develop a method for testing the potency of various substances used in the treatment for pernicious anemia. This phase has not been as yet thoroughly exhausted, but evidence has accumulated to show that desiccated hog stomach or potent liver extracts counteract the hemolytic action of sulphides. The hemoglobin values and the red cell counts begin to increase and the white cell counts and those of the reticulocytes to decrease after a simultaneous maintenance of dogs on the disulphide and the anti-anemic extracts. This did not occur as definitely and as regularly when the animals were maintained on extract devoid of activity or when inert liver ash was substituted for a potent extract.

## NUTRITIONAL MUSCULAR DYSTROPHY IN GUINEA-PIGS AND RABBITS. MARIANNE GOETTSCH and ALWIN M. PAPPENHEIMER, Columbia University.

An extensive and ultimately fatal dystrophy of the voluntary muscles was induced in guinea-pigs and rabbits by dietary means. The young animals at the time of weaning were given a natural diet, consisting of wheat bran, rolled oats, casein, skimmed milk powder, lard, cod liver oil, sodium chloride and calcium

carbonate, in which vitamin E had been destroyed by treatment with ethereal ferric chloride, and 3 or 6 cc. of orange juice daily.

Later experiments showed that the deficiency of vitamin E was not responsible for the disorder. Neither was the treatment with ethereal ferric chloride a factor. Scurvy, inanition and infection could be ruled out. Control animals on a diet of bran, oats, hay and greens had normal muscles. Rats were not susceptible to this dietary disease.

THE ACTION OF EPINEPHRINE IN DEPANCREATIZED ANIMALS. JESSE L. BOLLMAN, the Mayo Foundation, Rochester, Minn.

Depancreatized animals were maintained in good nutritive condition by large doses of insulin and appropriate feeding. Four days after the withdrawal of insulin, and fasting, specimens of muscle and liver were removed under ether anesthesia. Large amounts of epinephrine were then given over a period of twelve hours, at the end of which additional samples of liver and muscle were taken. The urine during the administration of epinephrine showed an excess of sugar that could not be accounted for by the nitrogen excreted, and a small amount of lactic acid was excreted. The glycogen content of the liver showed little change, and an increase in dextrose content was usually noted. The dextrose content of the muscle showed few changes, but the glycogen content of the muscle was reduced during the administration of epinephrine to very low values (from 20 to 40 mg. for each 100 Gm. of muscle). In all experiments the dextrose excreted in excess of D/N 2.8 was almost identical in amount with the loss of glycogen from the muscle. No evidence of the formation of dextrose from other substances (fat) was obtained.

MENSTRUATION AND FOCAL REACTIONS. W. F. PETERSEN, LLOYD ARNOLD and MARGARET MILLIKEN (by invitation), the University of Illinois.

Focal reactions in areas of either acute or chronic inflammation or in areas the seat merely of cellular dysfunction commonly present clinical manifestations. Indeed, not infrequently the entire clinical picture is dominated by the focal reaction.

The biologic alterations associated with the menstrual cycle are apparently frequently associated with the inception of such focal reactions, which may become manifest in the skin or mucous membranes, in the internal organs, in the central nervous system, etc.

There is a further importance that attaches to focal reactions inasmuch as we make use of them therapeutically in nonspecific therapy, and they probably play a rôle in the normal processes of healing and recovery, particularly in chronic infections. It is for this reason that the authors have studied in considerable detail the chemical alterations that occur during the course of the menstrual cycle and present a series of charts in which correlating observations have been made on the emotional status of the patient, the disinfecting power of the skin, the blood calcium, potassium, chlorides, sugar, cholesterol, phosphates, carbon dioxide content, hydrogen ion concentration, protein content, the leukocyte count, as well as basal metabolism, and such manifestations of the skin as the intracutaneous reaction to histamine and epinephrine, the dermographic reaction time, blister permeability, etc.

For the focal reaction that occurs in tuberculosis, the importance of the diminution in cholesterol, of the deficit of alkali and of the great increase in permeability of the capillaries that occur in the premenstrual phase would appear to be of considerable significance. Presumably the premenstrual phase is unfavorable so far as resistance to tuberculosis is concerned, while the chemical conditions that obtain in the postmenstrual period seem to be favorable toward healing and recovery.

For a syphilitic infection the opposite is probably true.

THE AVAILABILITY OF THE CALCIUM RESERVE OF THE SPONGY TRABECULAE AND COMPACT BONE IN EXPERIMENTAL HYPERPARATHYROIDISM. HENRY L. JAFFE, AARON BODANSKY and JOHN E. BLAIR (by invitation), Hospital for Joint Diseases, New York.

We have shown that young and adult guinea-pigs are susceptible to the effects of single and repeated doses of parathormone; the adult guinea-pigs show smaller changes in serum calcium and phosphorus and less resorption of bone than the younger animals. Histologic studies of the bones of guinea-pigs with acute or chronic experimental hyperparathyroidism have brought out the fact that the spongy trabeculae of the epiphyses are the least susceptible to decalcification. We have been unable to confirm the conclusions of Bauer, Aub and Albright that the bone trabeculae are more easily depleted than the cortex and are the source of labile calcium reserve. In young animals before closure of the epiphyseal cartilage plates the greatest degree of decalcification occurs in the metaphyses, at the costochondral junctions, and in the cortices of the ribs and long tubular bones. In older or in adult guinea-pigs the diaphyseal cortex is most involved. The trabeculae of the epiphyses showed no evidence of resorption in any of our animals except in one adult guinea-pig given 2,580 units of parathormone in doses pyramided over a period of forty-eight hours.

RENAL DENERVATION: I. EFFECT OF SNAKE VENOM. II. EFFECT OF REPEATED CHILLINGS. G. MILLES, E. F. MÜLLER and W. F. PETERSEN, the University of Illinois.

Accurate delineation of the renal vascular bed was obtained by the antemortem injection of 25 per cent solution of bismuth oxychloride into the abdominal aorta above the origin of the renal arteries. The kidneys were then removed, and x-ray photographs of them were made.

When this method was applied to animals on which unilateral renal denervation had been performed two or more weeks before, it was found that the injection of snake venom caused much more profound changes in the denervated than in the normal renal vascular tree, as demonstrated by immediate injection of the kidneys. From this it is concluded that the innervation of the kidney, by vasoconstriction, protects the organ from exogenous toxins.

On applying the same method for the demonstration of the renal vascular bed, it was found that daily chilling repeated for periods up to six months resulted in a marked decrease in the size of the vascular bed in the normal kidney as compared with the denervated kidney. The conclusion is drawn from this that vasoconstriction of the kidney is obtained by chilling and when repeated over a period of time results in definite vascular damage.

THE BONE MARROW IN AGRANULOCYTOSIS. RAPHAEL ISAACS, the University of Michigan.

In a patient who died from a severe infection with necrosis of the skin of the scrotal and perineal region, the blood showed a practically complete disappearance

	Sternal Marrow, per Cubic Millimeter	Vertebral Marrow, per Cubic Millimeter
Total cells .....	1,062,000	834,000
Red blood cells.....	666,000	504,000
Nucleated cells (all types).....	396,000	330,000
Polymorphonuclear cells .....	0	0
Metamyelocytes .....	0	0
Myelocytes .....	0	0
Myeloblasts .....	0	0
Eosinophils ("lymphoid") .....	29,526	37,635
Endothelial cells .....	62,526	46,315
"Lymphoid" cells .....	255,316	205,525
Erythroblasts .....	48,632	40,525
Megakaryocytes .....	Present	Present

of polymorphonuclear neutrophil leukocytes. On the day of death the white blood cell count was 700 per cubic millimeter. During the four weeks of illness the febrile reaction was marked, the temperature ranging from 102 to 104.5 F. The

bone marrow from the sternum and a vertebra, taken at autopsy, was very red, and the bone trabeculae were prominent. The cells were counted by drawing up fresh marrow to the "5" mark of a Sahli hemoglobin pipet, and diluting twelve times with blood serum and further diluting with Hayem's solution as in making a red blood cell count. The nucleated cells and red blood cells were enumerated separately. The remaining suspension of cells and serum was used to make cover glass films for differential cell counts (Wright's stain). The table shows the quantitative data.

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## CHICAGO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, April 13, 1931*

JOSEPH A. CAPPS, *President, in the Chair*

### UREMIC DERMATITIS. S. R. ROSENTHAL.

The characteristic macroscopic change of the skin in uremia is a "uremic frost." This occurred in thirty-three of fifty-six patients with uremia. In forty cases in which the skin was studied histologically there was a gradual atrophy of the skin directly proportional to the duration of the disease. This atrophy is explained on the basis of a constant saturation of the skin with urea (determined by the xanthidrol reaction), which interferes with the afferent and efferent circulations. Urea is not toxic, and it is found in the skin in direct proportion to the concentration of urea in the blood.

This complete report appears in *Arch. Dermat. and Syph.* **23**:934 (May) 1931.

### THE EARLY CELLULAR REACTION TO HUMAN TYPE TUBERCLE BACILLI IN THE LUNG OF THE RABBIT. ARTHUR J. VORWALD.

A previous study of the histopathologic reaction of the tissue of the lung in normal animals of varied species, at intervals of one day, one week and one month after an intravenous injection of a known amount of human type tubercle bacilli, demonstrated that in order to differentiate cell types partaking in the reaction intervals of less than twenty-four hours should be studied. My purpose is to describe the cellular response occurring in the lungs of normal rabbits at intervals of from one hour to one month after inoculation, stressing particularly the rôle of the polymorphonuclear leukocyte.

Rabbits were given intravenous injections of 0.1 mg. of human type tubercle bacilli per kilogram of body weight. The animals were killed at selected intervals after inoculation, and the lungs were immediately fixed in situ by the intratracheal injection of Zenker's solution of formaldehyde. The tissues were embedded and sectioned in celloidin. The tubercle bacilli were stained with carbol fuchsin, and for counter stain hematoxylin eosin-azure was used in one series and hematoxylin g-azure in another.

During the earliest stages, the first cells to react against the tubercle bacilli are the polymorphonuclear leukocytes, which encompass and phagocytose the organisms. Such foci invariably contain bacilli. This reaction is confined at first to the small capillaries, and then extends to the adjoining alveolar walls and spaces. Within fourteen hours, the polymorphonuclear leukocytes with and without tubercle bacilli are phagocytosed extensively by the mononuclear exudate cells, which increase in number to the exclusion of the leukocytes. The tubercle bacilli thus are liberated in the cytoplasm of the exudate cell. At stages later than eighteen hours, the mononuclear exudate cells with and without bacilli dominate the reaction so that at one month they constitute by far the majority of the cells in the typical tubercle. At this stage most of the mononuclear exudate cells have an oval, vesicular nucleus and a large, slightly foamy, pale blue cytoplasm. There is no selective localization of tubercle bacilli in the lymphoid aggregates. Almost always the bacilli lodge in the alveolar walls and there set up an inflammatory reaction.

Throughout all stages the absence of mitotic figures in the cellular reactions is striking.

I have reached the following conclusions:

The earliest cells responding to an intravenous injection of tubercle bacilli in rabbits are the polymorphonuclear leukocytes.

This reaction is inflammatory and specific. The polymorphonuclear leukocytes phagocytose the tubercle bacilli and play a great rôle in the early localization of these organisms.

Mononuclear exudate cells later dominate the cellular response, phagocytose and digest the polymorphonuclear cells and engulf their contained tubercle bacilli.

There is no primary localization of tubercle bacilli in the lymphoid aggregates of the rabbit's lung.

#### HEMORRHAGE AND "SHOCK" IN TRAUMATIZED LIMBS: TOTAL, FREE AND BOUND WATER CHANGES OF BLOOD AND MUSCLE. WILLIAM ROBINSON and ELOISE PARSONS.

The water content of the blood and muscles, in respect to both total water and water in the "free" and bound state, has been studied in animals under the influence of hemorrhage, histamine and trauma. The free water is that which freezes at  $-20^{\circ}\text{C}$ ., as determined by the "heat of fusion of ice" method of Rubner. The total water is determined by drying the material until a constant weight is obtained. The difference is termed bound water. The method is such that small samples can be analyzed, so that frequent determinations during the course of an experiment can be made and changing conditions can be studied.

The observations show that with hemorrhage there is a compensatory transfer of water chiefly in the "free" state from the muscles to the blood. In "histamine shock" the fall of blood pressure is associated with, and perhaps the result of, a transfer of water from the blood to the muscles. In "shock" produced by severe trauma to the leg (produced by blows from a padded hammer), the evidence indicates that with minor trauma there is in some cases a loss of fluid from the blood to the muscles associated with a slight fall in blood pressure, and that with major trauma the changes are those of hemorrhage in that there is a dilution of the blood remaining in the general circulation, at the expense of water withdrawn from the muscles.

The findings in this study are in support of the work of Parsons and Phemister, Blalock and others, which indicates the importance of the loss of blood in traumatized tissue in causing the fall of blood pressure after severe trauma.

#### SQUAMOUS CELL CARCINOMA IN A DERMOID CYST OF THE OVARY. P. A. DELANEY.

Dermoid cysts constitute about 10 per cent of all ovarian tumors, and in about 1 per cent malignant changes occur, usually carcinoma. In 1929, Masson and Ochsenhirt (*Surg. Gynec. Obst.* 48:702, 1929) reported three dermoid cysts with squamous cell carcinoma from the Mayo Clinic, their contribution bringing the total to thirty-six.

A white woman, aged 60, entered the Englewood Hospital, Chicago, because of pelvic pain of six or seven years' duration, which had become progressively worse especially during the three months before admission. On physical examination, a pelvic tumor was found. Operation revealed a large cyst containing about 1 quart of creamy fluid and large amounts of hair. The base of the cyst was adherent to unrecognized structures deep in the pelvis and consisted of friable tissue that appeared malignant. The abdominal wound never completely healed, and a fistulous opening persisted until the patient's death nine months later with symptoms of recurrence of the tumor. She died away from the city, and autopsy was not performed.

The emptied cyst weighed 156 Gm. The wall of the cyst, away from the base, where it was from 2 to 3 mm. thick, had a stratified squamous epithelial lining with frequent transitions to pseudostratified and simple columnar, partly

ciliated epithelium. Among the simple columnar epithelium were theca cells with specific mucin reactions. The subepithelial connective tissue stroma contained hair follicles with sebaceous glands, sweat glands and smooth muscle cells. Tissue from the base of the cyst showed extensive accumulations of anaplastic squamous epithelium with frequent "pearl" formation. Numerous large areas of necrosis, as well as many giant cells, involved both the stroma and the malignant tumor. In some of the sections with extensive carcinoma, cortical ovarian tissue stroma was identified. Sections removed from the inner lining of the cyst at its base, near where ulceration was present, had a gradual transition from normal to malignant, stratified, squamous epithelium.

#### MIXED TUMOR OF THE SCALP. P. A. DELANEY.

In 1930, Dr. Erb of the Konigsberg Policlinic published a lengthy article entitled "Rare Mixed Tumors of the Scalp" (*Beitr. z. klin. Chir.* **149**:617, 1930). He reported the first one seen in his clinic and contributed the fifth to the literature. His bibliography contains no reference to American, English or French authors.

A white man, aged 59, went to his physician for the removal of a small tumor over the left parietal prominence. He said that in 1915 or 1916 he had bruised the scalp. The injury healed without a scar, but about six months after the accident a barely perceptible mass appeared which grew slowly but progressively. Surgical removal was sought largely for cosmetic reasons.

A preoperative diagnosis of sebaceous cyst was made, but a solid tumor closely adherent to the overlying scalp was found. The surgical wound bled profusely and was closed without assurance that all of the tumor had been removed. The part removed was firm, white and yellow tissue weighing 1 Gm. and was 15 by 10 by 7 mm. It was fixed in Bouin's fluid.

Histologic preparations stained by various methods had a narrow fibrous connective tissue capsule. Epithelial tissues as intercommunicating and free tubulo-alveolar structures formed most of the section and frequently had a lumen content of individual round to oval cells that resembled those of the layers of the inner wall, their cytoplasm being homogeneous and acidophilic; the basal cell layer or layers had an intracellular content of fibrillae similar to that of the excretory duct of related marginal groups of sweat glands, or that of so-called myo-epithelial cells common to the sweat and mammary glands. In many places there was a proliferation of basal fibrillar cells into the adjoining stroma, while in other locations the inner nonfibrillar cells had proliferated and invaded the stroma to induce marked changes and become greatly altered in structure. The stroma was homogeneous and stained intensely red with mucicarmine, while most of the cells were polymorphic and vacuolated, singly or in groups. No daughter capsules were evident, but the stroma about the isolated or grouped cells frequently took a deeper stain, giving the impression of a capsule. On superficial examination, this latter tissue had cartilaginous characters, but in fact it was stroma tissue with changes accompanying the advance of invading epithelial cells of a specific type. The tumor did not have stellate myxoblasts. There were small isolated areas resembling intracanalicular fibro-adenoma of the breast and groups of tumor cells in the lumen of blood vessels without connection with the endothelial lining. There was no mitotic activity.

Four months after operation, the patient had a healed surgical scar and two small subcutaneous masses about 2 and 4 mm. in diameter, respectively. Five or six hair shafts projected over them, although the patient had an advanced stage of baldness.

This tumor illustrates the transformation of collagenic connective tissue stroma into a pseudocartilaginous matrix by the invasion of epithelial cells. This suggests that a similar change takes place in mixed tumors of the salivary glands. Although the structure of the adenoma favors origin in sudoriparous glands, the possibility of the participation of sebaceous glands or hair follicles in its formation is not disproved.



THE ASSOCIATION OF PSEUDOHERMAPHRODITISMUS MASCULINUS EXTERNUS WITH  
ADRENAL HYPERPLASIA AND VASCULAR HYPERTENSION. CLAIRE E.  
HEALEY and CHESTER C. GUY.

This article will be published in full in a later issue of the ARCHIVES.

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*Regular Monthly Meeting, May 11, 1931*

PAUL R. CANNON, *President pro tem., in the Chair*

THE IMMUNOLOGIC SPECIFICITY OF ANTIGENS OF THE BRAIN. JULIAN H.  
LEWIS.

Many attempts have been made to demonstrate for the various organs of the body an immunologic specificity that is comparable to the specific character of their functions, anatomy and chemical composition. The existence of organ-specific antigens permits the immunologic differentiation of the different organs within the same species and establishes a relationship of the same organ in different species. The classic example of such an organ specificity is that of the crystalline lens. An absolute differentiation of this organ from all other organs in the body can be made by appropriate immunologic reactions, and as a corollary, no differentiation can be made between the crystalline lens of any species. This complete organ specificity also carries with it the ability to form iso-antibodies. An animal can be sensitized and shocked with its own lens. As to other organs of the body, a slight and inconstant differentiation can be made within the species. This has been found to be particularly true in comparisons of the sex organs with other organs, but no relation has been found between sex organs of different species.

Recently, Witebsky reported that the brain can be placed in the same class as the crystalline lens, since it contains antigens that show absolute organ specificity. The only difference between the crystalline lens and the brain is that the specific antigen of the latter is reported to be lipin. Because of the importance of this contribution, I have repeated and extended Witebsky's experiments in order to verify them.

Rabbits immunized with a suspension of rat, beef or guinea-pig brain form anti-serums that react not only with suspensions of brains of other mammals, but also with those of birds, fish and reptiles. Alcoholic extracts serve as antigens just as well as do the suspensions. By saturation experiments, some differentiation can be made between these interacting antigens of the brain, since by saturation experiments all antibodies for a heterologous antigen can be removed without removing the antibodies for the homologous antigen of the brain.

GAUCHER'S DISEASE. ERNEST KRAFT and HENRY F. HOOKER.

An unmarried woman, aged 25, with a markedly enlarged spleen, had lost weight and strength for several months. The spleen, which was removed surgically, measured 31.2 by 21.6 by 14.4 cm. and weighed 6,800 Gm. The histologic structure was that of Gaucher's disease. Following splenectomy, the patient improved markedly in health. The father and a brother of the patient are known to have had enlargement of the spleen.

CONGENITAL DIAPHRAGMATIC HERNIA ASSOCIATED WITH AN ACCESSORY LUNG.  
CHESTER C. GUY and GEORGE RAND.

A full-term female infant lived one hour after birth. A diaphragmatic hernia on the left bulged into the chest and occupied three fourths of the left half and contained the stomach, the small bowel, the cecum, the ascending colon, the pancreas and the spleen. In the lower portion of the left pleural cavity, behind and medial to the hernial sac, was an accessory mass of lung tissue, 4 by 2 by

2.5 cm., attached medially to the upper surface of the diaphragm at the edge of the hernial opening by a pedicle 8 by 8 mm. The arterial blood supply was chiefly through a branch from the thoracic portion of the aorta. Microscopically, the structure of the tissue was that of fetal tissues of the lung with atelëctasis.

FAMILIAL CONGENITAL ATRESIA OF THE INTESTINE. ERIC A. FENNEL.

A secundipara, aged 27, was delivered of a male child with a distended abdomen. The anal opening of the child was patent, but an obstruction of the rectum was found 2 inches (5 cm.) above. Eight hours after birth, with the patient under spinal anesthesia, laparotomy released more than a pint of serofibrinous exudate and demonstrated extensive adhesions between the abdominal viscera. The rectum was small, and the sigmoid colon had a diameter of 9 mm. and seemed solid. A tube was inserted into the rudimentary lumen, and the laparotomy wound was closed. The child died seven hours later (fifteen hours after birth). Cultures of the peritoneal exudate were sterile. There were no changes in the esophagus, lungs, heart, pleurae, gallbladder, bile ducts, kidneys, ureters and urinary bladder. A generalized fibrinous exudate covered the viscera of the abdomen. The lower portion of the duodenum had a thin wall and an empty dilated lumen. The jejunum and ileum were distended with meconium, soft in the upper loops and firm in the lower loops. The wall of the small bowel was thin. The cecum was large; the colon was firm and had a diameter of 9 mm. The wall of the first 7 cm. was thin; the lumen was 7 mm. in diameter and contained brown meconium. Beyond this portion, the lumen of the colon was a small cleft which disappeared in the rectum. Histologic preparations of the small bowel had no noteworthy changes. Those of the colon had the usual tissue structures. There were no inflammatory reactions.

On the third day after birth, the brother of this child, who was born to the mother thirteen months before her second confinement, had symptoms suggesting pyloric stenosis. The distal 8 inches (20.32 cm.) of the small bowel and the entire colon had a diameter of 1 cm., and the cecum was small. There were no tortions or thrombosed vessels. No congenital anomalies are known in the families of the father and the mother of these infants. Some hereditary factor seems concerned with the malformations.

## Book Reviews

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DIE SEXUELLEN ZWISCHENSTUFEN. By RICHARD GOLDSCHMIDT, Professor und Direktor am Kaiser Wilhelm-Institut für Biologie im Berlin. Price, 45 marks. Pp. 520, with 214 illustrations. Berlin: Julius Springer, 1931.

The problem of the unusual conditions of the reproductive system, which are of far more frequent occurrence than is known by even the most scientific investigators, has at once a remarkable attraction for the general scientist, biologist, animal breeder and clinician. The once generally accepted idea of the stability of sex and its concreteness—entire maleness or femaleness—is gradually giving way to an appreciation of the plasticity of sex, in extreme cases of which an organism may at one time display the female characters and produce ova and at a later time develop the characters and psyche of the male and even produce spermatozoa. Between the two extremes of maleness and femaleness there exists a vast field of sexual intermixtures—intersexes, sex intergrades, hermaphrodites and gynandromorphs—in which all stages of the reproductive organs and associated external and internal structures of both sexes are represented in the same organism.

Professor Goldschmidt is a leading authority on the subject of intersexuality by virtue of practically continuous investigation in this field for more than twenty years. In this book he not only summarizes his own extensive investigations dealing with the crossing of races, embryology, cytology and genetics as they have been means of analyzing the sexual conditions presented, but also gives an exhaustive review of the facts and theories of intersexuality presented by other investigators and covering the entire animal kingdom.

After carefully distinguishing between such types of sex intermixtures as the true intersexes and gynandromorphs Goldschmidt proceeds to analyze the approach to the study of the problems and properly emphasizes the necessity of knowing the embryologic, anatomic, cytologic and genetic phases of the particular individual or group under investigation. His own extensive contributions on the peculiarities that have arisen in *Lymantria*, the gypsy moth, in which for example a given male when crossed with female (a) of one race produces offspring that are normal, whereas when crossed with female (b) from another race produces offspring all, or definite proportions, of which show reproductive systems or other sex characters of mixed type and covering the complete range from one sex to the other, are explained by different intensities, valences or strength of the individual sex determiners. Thus a race may be out of balance in its sex determiners with a different race, and the progeny arising with the imbalance of determiners for maleness or femaleness may show an intermediate or mixed development that may occur at different critical periods in development. The relative period with which the imbalance becomes effective in development accounts for the different degrees of intermixture. A zygotically determined female offspring, for example, may show this critical point sufficiently early in development as to develop completely into a male and so function. Genetic analyses have revealed the true nature of such offspring. Following the impressive array of evidence in support of his quantitative theory of sex, now generally recognized as a fundamental biologic principle in the determination of sex, Goldschmidt reviews the known facts of intersexuality demonstrated among fish, amphibia, birds and mammals, including man; he thus offers a common ground for an explanation of intersexuality among all the animal groups, invertebrates as well as vertebrates, but as sharply as possible he distinguishes between genetic and hormonal influences as they operate in the life of the organism.

The reader is impressed with the general organization and the handling of the vast complexities brought under consideration. Much more than half of the book deals with intersexual conditions found among the vertebrate group. The book is excellently illustrated with both original and recently published illustrations and with instructive explanatory diagrams. It is notable that a great deal of the literature of 1930 forms an integral part of the book, hence it is well up to date on the findings in this particularly interesting field of research.

THE PHYSICIAN OF THE DANCE OF DEATH. A HISTORICAL STUDY OF THE EVOLUTION OF THE DANCE OF DEATH MYTHUS IN ART. By ALDRED SCOTT WARTHIN, PH.D., M.D., LL.D., Professor of Pathology and Director of the Pathological Laboratories in the University of Michigan, Ann Arbor, Mich. Price, \$7.50. Pp. 142, with 92 illustrations. New York: Paul B. Hoeber, Inc., 1931.

This book is a reprint with additions and corrections of articles in *Annals of Medical History*. Since his student days the author has felt a special interest in representations in art of human death. During the years scattered hours of leisure were given to the study of writings and works of art representing the thoughts about death by European peoples during the past six centuries. Little by little the author gathered a unique bibliographic collection concerning the Dance of Death, and this collection is the basis of the book.

The physician in the Dance of Death motive, in which the personified human skeleton stands for the process of death, is followed from the late fourteenth century to the present time in scholarly and artistic discussion. The introduction deals with the beginnings and early significance of the Dance of Death. The discussion of its further evolution is divided logically as follows: The period of the great wall paintings; the pre-Holbein manuscripts, block books and the Incunabula; Holbein; the imitators of Holbein; the period of caricature; the modern Dance of Death. Most of the illustrations reproduce various pictures of Death and the Physician. The physicians of the past are seen under conditions that reflect their ways of practice, their character and social standing and the contemporary state of medical knowledge. The anatomist is represented in these figures, but the pathologist seems to have been overlooked. At present, the physician himself seems to be stepping out of the picture.

"The Dance of Death idea is as immortal as the life of the race; but in each new period of human thought it will express itself in new form corresponding to the predominant philosophy of that time. From the primitive wall paintings of the Middle Ages as marking the period of religious superstition, to the intellectual creation of Holbein as characterizing the Renaissance, and following this the gradual decrescendo through the Rococo period to modern times, the Dance of Death motive has followed a definite evolution which now awaits the artist of the modern period, who will express adequately in some art form the changed philosophy of the twentieth century toward Death; the Totentanz mythus awaits a new birth."

The work is a choice product of the wise use of leisure hours in the pursuit of a fascinating hobby.

ANTIGENS IN THE LIGHT OF RECENT INVESTIGATIONS. By OLUF THOMSEN, Professor of General Pathology in the University of Copenhagen. Translated from the Danish by Hans Andersen, M.D. Pp. 187. Copenhagen, Denmark: Levin and Munksgaard, 1931.

The book is divided into three chapters. The first chapter deals with the definition of antigens and antigenic functions. In the second chapter, which is the longest, the different kinds of substances that may act as antigens are discussed. First come the proteins, the natural as well as the artificially altered;

then the lipoids, including the heterogenetic antigens, the carbohydrates and their rôle in the specificity of bacterial species and bacterial types; the so-called chemospecific antigens, and finally, the true bacterial toxins. The third chapter considers certain special problems in the light of the progress in the study of antigens: the nature of the Wassermann reaction, lipoid anaphylaxis, idiosyncrasy (synonym for hypersusceptibility, sensitization and allergy) and bacterial allergies.

The author has succeeded well in presenting the main advances of the recent investigations of antigens. The book will be particularly helpful in reviewing the present knowledge of haptenes, carbohydrates, lipoids and chemospecific antigenic substances and their rôle in immune reactions, in specificity and in allergy. The advances in the understanding of the rôle of haptenes may lead to a more satisfactory interpretation of certain forms of hypersusceptibility. Technical details of the preparation of antigenic substances or of tests of various kinds are not discussed. The great problem of how antigens act in the body in the production of antibodies is hardly touched. But then it is true that no signal advances have been made in respect to the fundamental problem of the production of antibodies since 1920, which is the period with which the book is particularly concerned. The course and distribution of antibodies in the immunized organism are not considered.

The translation is fairly satisfactory; in the main the meaning is clear, but at times the language is cumbersome and occasionally one notices peculiar turns, e. g., "regarded after this theory" in place of interpreted in accord with this theory; in other instances the word after is used in the sense of because; "the hypothesis seems constructed" in place of forced or labored. In the section on idiosyncrasy (allergy, hypersusceptibility), idiosyncratic is used freely as synonymous with allergic, hypersusceptible and hypersensitive. An index would have been helpful. The German word "Schlepper," which is used by Sachs and others to describe the more or less mechanical rôle of foreign serum in establishing the antigenic effect in vivo ascribed to certain lipoids, has not received a satisfactory English equivalent. The suggestion is ventured that "conveyor" or "carrier" seems to meet the need. The general quality of the subject matter of the review is praiseworthy. The topics presented are discussed satisfactorily in a critical but constructive and helpful spirit.

THE FACTOR OF INFECTION IN THE RHEUMATIC STATE. By ALVIN F. COBURN, Resident Physician of the Presbyterian Hospital in the City of New York. Price, \$6. Pp. 288, with 48 illustrations. Baltimore: Williams & Wilkins Company, 1930.

Most of the material for this book was obtained from a group of 162 patients with rheumatic fever and rheumatic endocarditis studied over a period of years. The author has made a composite picture of all the clinical phenomena observed in this group, which he calls rheumatic disease. *Streptococcus haemolyticus* was found to be the predominant organism in the upper respiratory tract at the onset of rheumatic attacks in almost all patients. The author observed that hemolytic streptococci are seldom found in the throats of people living in Porto Rico, and that rheumatic fever rarely occurs in the tropics. Ten patients with persistently active rheumatic disease were sent to Porto Rico for six months. They showed considerable improvement, and the hemolytic streptococci disappeared from their throats. As soon as the patients returned to the United States, the same organisms reappeared in their throats and the rheumatic attacks began again. Tests of the skin with the nucleoprotein of *Streptococcus haemolyticus* showed decided reactions in almost all patients with active rheumatic disease. Case histories, most of which are repetitious; occupy a large portion of the book. A concise article would cover the material of importance.

DIE HISTOGENESE EKTO-MESO-DERMALER MISCHGESCHWÜLSTE. EIN BEITRAG ZUR FRAGE DER ORGANISATORENWIRKUNG (SPEMANN) BEIM PATHOLOGISCHEN WACHSTUM. By PROF. DR. PAUL SCHÜRMANN, DR. MED. HANS PFLÜGER and ZAHNARZT DR. WILHELM NORRENBROCK. Price, 11.50 marks. Pp. 94, with 79 illustrations. Leipzig: Georg Thieme, 1931.

This attractive monograph supports the thesis that odontomas of the hypophyseal duct and mixed salivary tumors are primary epithelial tumors, the part taken in their growth by mesenchymal elements being secondary and determined by the organizing influence of the oral epithelium. The senior authors (Pflüger and Schürmann) describe in detail two instances of complicated odontomas of the hypophyseal duct and discuss their morphologic and genetic relations to other tumors of the duct. Norrenbrock considers the histogenesis of mixed tumors of the parotid glands. Both parts have many and excellent illustrations. While the tumors under consideration are mixed in the descriptive sense, they are interpreted by the authors as being essentially and genetically epithelial. The complexity of their structure is ascribed to the many potentialities of the oral ectoderm. The mesenchymal elements are held to develop under and in response to an organizing influence on the part of the epithelial cells, but just how this influence is brought into action is not explained. The morphologic descriptions are thorough and authoritative.

## Books Received

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DIE LEBENS VorgÄNGE IM NORMALEN KNORPEL UND SEINE WÜCHERUNG BEI AKROME GALIE. Von Professor Dr. J. Erdheim a.o. Professor an der Universität Wien. Price, 18 marks; bound, 19.60 marks. Pp. 160, with 31 illustrations. Berlin: Julius Springer, 1931.

LEPTOSPIROSIS IN THE ANDAMANS WITH AN APPENDIX ON THE PRESENT KNOWLEDGE OF LEPTOSPIRAL INFECTIONS. By Lieut.-Col. J. Taylor, D.S.O., M.D., D.P.H., I.M.S., and Amar Nath Goyle, M.B., Ph.D. (from the Pasteur Institute of Burma, Rangoon), Indian Medical Research Memoirs no. 20. Price, 6-0-0 rupees or 8 shillings. Pp. 190. Calcutta: Thacker, Spink & Company, 1931.

THE RENAL LESION IN BRIGHT'S DISEASE. By Thomas Addis, Professor of Medicine, Stanford University Medical School, San Francisco, and Jean Oliver, Professor of Pathology, The Long Island College of Medicine, Brooklyn; formerly Professor of Pathology, Stanford University, San Francisco. Price, \$16. Pp. 650, with 181 illustrations (some in color), and large table. New York: Paul B. Hoeber, Inc., 1931.

WILLIAM STEWART HALSTED, SURGEON. By W. G. MacCullum. Introduction by Dr. W. H. Welch. Price, \$2.75. Pp. 246. Baltimore: Johns Hopkins Press, 1931.

STREPTOCOCCIC BLOOD STREAM INFECTIONS. By GEORGE E. ROCKWELL, M.A., M.D., Associate Professor of Bacteriology, College of Medicine, University of Cincinnati; Member of Senior Medical Staff, Bethesda Hospital; Assistant Director of Bacteriological Service, General Hospital of Cincinnati. Price, \$1.75. Pp. 73. New York: The Macmillan Company, 1931.

CLINICAL DIAGNOSIS BY LABORATORY METHODS. By James Campbell Todd, Ph.B., M.D., Late Professor of Clinical Pathology, University of Colorado, School of Medicine; and Arthur Hawley Sanford, A.M., M.D., Professor of Clinical Pathology, University of Minnesota (Mayo Foundation); Head of Section on Clinical Laboratories, Mayo Clinic. Seventh Edition. Price, cloth, \$6 net. Pp. 765, with 347 illustrations, 29 in color. Philadelphia: W. B. Saunders Company, 1931.

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## REACTIONS OF THE BONE MARROW IN EXPERIMENTALLY INDUCED THROMBOCYTOSIS \*

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### I. REVIEW OF PREVIOUS WORK AND ANALYSIS OF THE PROBLEM OF THE ORIGIN OF BLOOD PLATELETS

Since Wright's<sup>1</sup> theory of the origin of blood platelets from megakaryocytes was first proposed in 1906, it has been the subject of a lively dispute. Although the theory is now widely accepted, apparently valid and reasonable evidence against it continues to be produced. Moreover, much of the experimental work in support of the theory is subject to one serious criticism, namely, that the studies of the bone marrow after experimental alteration of the platelet level were inadequate. I have therefore undertaken an investigation of the bone marrow in certain cases of thrombocytosis.

In order to avoid the errors that appear in much of the previous work, an analysis has been made of the evidence presented on both sides of the question and of the methods of other investigators. This includes, first, observations on the bone marrow in certain clinical conditions; second, observations on the blood and the endothelium in clinical and experimental thrombocytosis and thrombopenia; third, a summary of important experimental investigations, and fourth, a résumé of the observations of other authors on the variable appearances of the megakaryocyte in normal and pathologic states. The criticisms arising from this analysis led to a consideration of the ideal method of approach to any investigation of Wright's theory.

### BONE MARROW IN CERTAIN CLINICAL CONDITIONS

Clinical observations bearing on Wright's theory include, first, those of Wright himself,<sup>2</sup> who pointed out that both the number of circulating platelets and the number of megakaryocytes in the bone marrow are reduced in pernicious anemia and in lymphatic leukemia, and that both are increased in other anemias and in myelogenous leukemia. Selling,<sup>3</sup>

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1. Wright, J. H.: *Virchows Arch. f. path. Anat.* **186**:55, 1906.

2. Wright, J. H.: (a) Footnote 1; (b) *J. Morphol. & Physiol.* **21**:263, 1910.

3. Selling, L.: *Bull. Johns Hopkins Hosp.* **21**:33, 1910.



in cases of purpura due to benzol poisoning, found a great reduction of platelets and an absence of megakaryocytes in the aplastic bone marrow at autopsy. Ogata <sup>4</sup> in a case of myeloid leukemia with increased platelets found myeloid metaplasia of the kidney and liver and megakaryocytes present in numbers in these locations. Katsunuma <sup>5</sup> stated that increase or decrease of platelets and increase or decrease of megakaryocytes always go hand in hand. Dyke <sup>6</sup> corroborated Wright's observations on the platelet count and on the bone marrow in leukemias.

Not all of the evidence has been so decisive, however, for Bunting <sup>7</sup> reported definite increase in megakaryocytes in Hodgkin's disease, but no correlation between this increase and the platelet count, which was found to be variable. It should be noted that some of the megakaryocytes showed pyknosis and karyolysis, and may have been incapable of functioning in their platelet-forming capacity. This subject will be discussed later. Dyke, also, found that the megakaryocytes showed no constant changes in number in cases of purpura, one group of cases showing megakaryocytes present in normal and excessive numbers, and another showing an aplastic bone marrow with no megakaryocytes. Again it should be noted that in those cases in which megakaryocytes were numerous, they were abnormal, showing no cytoplasmic granules. Although he reported no platelet counts in these cases, it is now well known, since the appearance of Duke's work,<sup>8</sup> that the number of platelets is usually reduced in cases of purpura.

#### FORMATION OF PLATELETS OUTSIDE OF BONE MARROW

Further evidence in favor of Wright's theory has been adduced from such observations as the appearance of megakaryocytes in their entirety, or of large fragments of their cytoplasm, in the blood stream in certain cases of clinical and experimental thrombocytosis. Di Guglielmo <sup>9</sup> stressed this in cases of leukemia and cited a number of previous authors who had made the same observation; he found, also, certain indications of the formation of platelets in these cells. During exacerbations in cases of leukemia, Minot <sup>10</sup> found showers both of entire megakaryocytes and of large fragments of cytoplasm in the blood stream; at autopsy the capillaries of the lungs were crowded with these same structures, and an increase in platelets usually accompanied their appearance. Similar changes have been noted in experimental conditions. For

4. Ogata, S.: Beitr. z. path. Anat. u. z. allg. Path. **52**:192, 1912.

5. Katsunuma, S.: Folia haemat. **32**:29, 1925.

6. Dyke, S. C.: Lancet **2**:714, 1924.

7. Bunting, C. H.: Bull. Johns Hopkins Hosp. **22**:114, 1911.

8. Duke, W. W.: The Pathogenesis of Purpura Hemorrhagica, with Especial Reference to the Part Played by Blood Platelets, Arch. Int. Med. **10**:445, 1912.

9. di Guglielmo, G.: Haematologica **1**:303, 1920.

10. Minot, G. R.: J. Exper. Med. **36**:1, 1922.

example, Seeliger<sup>11</sup> found in the blood stream after the injection of peptone, not only large masses of the cytoplasm of megakaryocytes, in many of which the granules were clumped into small groups preparatory to the fragmentation of the cytoplasmic mass into platelets, but also chains of platelets ("Perlenketten"), complete except for the final separation of the intervening fine cytoplasmic thread. Sabin<sup>12</sup> had the good fortune to capture a megakaryocyte in a preparation of myelogenous leukemic blood in the warm stage, and observed it fragmenting into pieces that resembled in every detail normal platelets. Bunting<sup>13</sup> noted the common occurrence of large fragments of megakaryocytes and the great variation in the size of platelets in cases of Hodgkin's disease, in which, as has been stated, the count of megakaryocytes was always increased, although the platelet count tended at first to be high and subsequently low. He found, also, naked nuclei of megakaryocytes in the lung. Katsunuma<sup>5</sup> stated that emboli consisting of the nuclei of megakaryocytes always accompany an increase in platelets. Firket and Campos<sup>14</sup> found such emboli after experimental saponin poisoning in rabbits.

It may, perhaps, be worthy of note that a number of authors have suggested that some other cell than the megakaryocyte is capable of taking over the platelet-forming function when the megakaryocyte is rendered incapable, or when the demand for platelets is great. In a case of influenza with low platelet count and inhibition of the activity of the bone marrow due to toxic damage, Bunting<sup>15</sup> found every large lymphocyte in the smear showing azurophil granules grouped at the periphery and forming single platelets. Brown,<sup>16</sup> in experimental thrombopenia produced by the injection of alkaline hematin in rabbits, found platelets budding from hypertrophied endothelial cells and from mononuclear cells in the bone marrow and circulating blood. Cramer and Drew<sup>17</sup> observed platelets budding from endothelial cells in subcutaneous lymph nodes. Ferrata and Rinaldi<sup>18</sup> found platelets budding from monocytoïd cells which they believed to be young megakaryocytes.

#### EXPERIMENTAL INVESTIGATION OF WRIGHT'S THEORY

The investigation of the validity of Wright's theory has not, however, been content to rest on more or less isolated clinical observations.

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11. Seeliger, S.: *Folia haemat.* **29**:23, 1923.

12. Sabin, F. R.: *Bull. Johns Hopkins Hosp.* **34**:277, 1923.

13. Bunting, C. H.: (a) Footnote 7; (b) *J. Exper. Med.* **11**:541, 1909.

14. Firket, J., and Campos, E. S.: *Bull. Johns Hopkins Hosp.* **33**:271, 1922.

15. Bunting, C. H.: *Bull. Johns Hopkins Hosp.* **31**:439, 1920.

16. Brown, W. H.: *J. Exper. Med.* **18**:278, 1913.

17. Cramer, W., and Drew, A. H.: *Brit. J. Exper. Path.* **4**:271, 1923.

18. Ferrata and Rinaldi: *Folia med.* **1**:815, 839, 863 and 891, 1915.

An enormous amount of experimental work has been done. The usual method of approach has been the use of some experimental agent that reduces the number of circulating platelets, combined with a correlated study, more or less adequate, of megakaryocytes in the bone marrow. Such studies were made either during the time of reduction of the number of platelets or during the period of return to normal or during the compensatory increase that usually follows. Some of the more important investigations, most of them, though not all, corroborating Wright's theory, will be briefly presented in chronological order.

In 1909, three years after Wright's first publication, Bunting<sup>18b</sup> undertook to show that changes in the number of platelets are not immediately correlated with changes in the number of any other of the circulating elements; i. e., that platelets are not dependent for their origin on the circulating cells, but arise in the bone marrow from megakaryocytes. Bleeding of rabbits caused a fall in the red cells and platelets, but the platelets returned to normal before the red cells recovered. After injections of saponin, likewise, the platelets rose above normal long before the red cells recovered. Sterile inflammation induced by the subcutaneous injection of croton oil and turpentine and the intraperitoneal injection of aleuronat produced both thrombopenia and leukopenia, but the platelets exhibited both a compensatory increase and a return to normal while the leukocytes were still rising. These experiments showed the independence of platelets both from erythrocytes and from leukocytes. Bunting then counted the megakaryocytes of the bone marrow of the experimental and normal rabbits and found that when the number of platelets was for any reason increased above normal, the number of megakaryocytes was increased above 20 per square millimeter (the average normal), and conversely, when platelets were reduced, megakaryocytes, also, were reduced.

Ogata<sup>4</sup> (1912) found in rabbits that after bleeding the increase of platelets was accompanied by an increase of megakaryocytes of 100 per cent (140 per visual field, as compared with a normal of 70), that Wright's figures were increased in number, and that "Felderung," or the grouping of granules supposed to be preparatory to the breaking up of the pseudopods into platelets, and the pinching off of platelets singly were more frequently seen. Similar pictures were seen after the intravenous injection of staphylococcus cultures into rabbits.

Duke<sup>19</sup> (1912 and 1913), in an experimental study of purpura, produced variations in the platelet level in rabbits by the injection of diphtheria toxin, benzol and tuberculin, all toxic to bone marrow. By grading the dosages of the first two, more toxic, substances, he was

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19. Duke, W. W.: (a) Footnote 8; (b) Causes of Variation in the Platelet Count, *Arch. Int. Med.* **11**:100, 1913.

able to produce stimulation of the bone marrow with an increase in platelets, or destruction of the marrow with a decrease in platelets and the occurrence of purpura; in the latter case the megakaryocytes were almost completely absent.

Brown<sup>10</sup> (1913) found that alkaline hematin was highly toxic to both platelets and megakaryocytes. His finding of platelets arising from cells other than the megakaryocyte in this condition has already been mentioned.

A most ingenious method for the removal of platelets from the blood stream was devised by Ledingham<sup>20</sup> (1914 and 1915) and was used by him to produce experimental purpura. This work was amplified by Bedson<sup>21</sup> (1921 and 1923). An antiplatelet serum was produced in rabbits by injecting the platelets of guinea-pigs, and this proved to be highly destructive to platelets when reinjected into the guinea-pig. Bedson, who at one time (1923)<sup>22</sup> inclined to the older view that platelets are not formed from megakaryocytes, but are an independent genetic line, at first nucleated like the thrombocytes of lower forms, but losing their nuclei after the manner of mammalian red cells, undertook to discover (1925)<sup>23</sup> in which tissues or organs in the body platelets arise. Using as antigens lymph node, bone marrow, endothelial cells (large mononuclear cells obtained by inducing peritoneal exudate with old tuberculin and supposed to represent the reticulo-endothelial system), spleen and a quantity of blood equal to that contained in the spleen, he prepared antiserums from these antigens in the same manner as he prepared his antiplatelet serum. Of these, the serums prepared against bone marrow and spleen were highly specific against platelets. Since he had previously shown (1924)<sup>24</sup> that the spleen stores and destroys platelets, he reasoned that the specificity of the antispleen serum was due to the spleen's content of stored platelets. Therefore, the bone marrow produces platelets, being the only tissue other than the spleen in which they or their parent tissues are present in sufficient concentration to stimulate the formation of antiplatelet antibody when the tissue is used as an antigen. This conclusion led him to the investigation of the megakaryocytes. He found an increase in the number of megakaryocytes after the destruction of platelets by antiplatelet serum, and so was led rather reluctantly to an acceptance of Wright's theory.

Ferrata and Rinaldi<sup>18</sup> (1915) in a study concerned, chiefly, with the origin and form of the megakaryocyte, induced augmentation of the

20. Ledingham, J. C. G.: *Lancet* **1**:1673, 1914. Ledingham, J. C. G., and Bedson, S. P.: *Lancet* **1**:311, 1915.

21. Bedson, S. P.: *J. Path. & Bact.* **24**:469, 1921; **26**:176, 1923.

22. Bedson, S. P.: *J. Path. & Bact.* **26**:145, 1923.

23. Bedson, S. P., and Johnston, M. E.: *J. Path. & Bact.* **28**:101, 1925.

24. Bedson, S. P.: *Lancet* **2**:1117, 1924.

number of platelets by the use of pyrodine, pyrodine combined with bleeding, and lead, in the rabbit, dog, guinea-pig and mouse, and found increased numbers of megakaryocytes in the marrows of these animals.

Firket and Campos<sup>14</sup> (1922), using methods similar to those of Foa,<sup>25</sup> Bunting<sup>13b</sup> and Isaac and Möckel,<sup>26</sup> investigated the action of saponin on blood platelets, first, by allowing it to come in contact with platelets in vitro, and second by simultaneous counts from a vein of the ear, the splenic vein and the portal vein after injecting it into rabbits, proving that platelets are actually destroyed by the toxin. Saponin is toxic also to the bone marrow, producing hemorrhages and focal necrosis. This destruction is accompanied by myeloid metaplasia of the spleen and liver where megakaryocytes appear in large numbers and Wright's figures are conspicuous.

Fabricius-Möller<sup>27</sup> (1922) found that massive doses of x-rays produced a diminution in the number of platelets in guinea-pigs, and when the dosage was sufficient, irradiation was followed by a hemorrhagic diathesis and even by death. He observed disintegration of the megakaryocytes previous to the reduction in the number of circulating platelets.

Seeliger<sup>11</sup> (1923) used injections of peptone to produce thrombopenia. Studies of the bone marrow showed increased numbers of active megakaryocytes. The signs of platelet-forming activity in megakaryocytes he described as (1) the presence of abundant azurophil granules in the cytoplasm, (2) the grouping of these granules ("Felderung"), (3) the formation of pseudopods and (4) the grouping of the granules in the pseudopods in such a way as to form chains of platelets ("Perlenketten").

In a series of experiments on the effects of benzol on hematopoiesis in rabbits, Weiskotten, Wyatt and Gibbs<sup>28</sup> (1924) observed an initial rise in the platelet count, then a fall during the stage of degeneration of the bone marrow, and finally an increase, at which enormous numbers of megakaryocytes appeared in the regenerative marrow.

Cramer and Drew<sup>17</sup> (1923) found platelets reduced in rats suffering from a deficiency of vitamin A and recovery of the normal number as the animals recovered from their deficiency disease under the influence of cod liver oil. They reported that there was no evidence of megakaryocytes participating in this increase, but stated that platelets were found budding from endothelial cells in the subcutaneous lymph nodes.

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25. Foa, cited by Firket and Campos (footnote 14).

26. Isaac and Möckel, J.: *Compt. rend. Soc. de biol.* **87**:759, 1922.

27. Fabricius-Möller, J.: *Compt. rend. Soc. de biol.* **87**:759, 1922.

28. Weiskotten, H. G.; Wyatt, T. C., and Gibbs, R. F. D.: *J. M. Research* **44**:593, 1924.

A rather extensive investigation of Wright's theory was reported by Petri<sup>29</sup> (1925 and 1926). He first established the normal platelet count and the limits of its variation in a series of rabbits and made careful counts of the megakaryocytes in these animals; in addition, a differential study of the megakaryocytes established the ratio between granular and nongranular cells and the number of Wright's figures. He then used a number of the experimental agents reported on by other authors to produce variations in the platelet count. Finally, he devised a method of his own, called by him "ideal blood platelet bleeding," consisting of the removal of a relatively large quantity of blood, its fractional centrifugation to remove platelets and the return of all the blood constituents except the platelets, plus, necessarily, the citrate required to prevent coagulation during this procedure. Saponin reduced the numbers of platelets and megakaryocytes, as had been reported previously, but when the platelet level returned to normal the megakaryocytes not only remained low, but were further reduced. Large hemorrhages reduced the platelet count; frequently repeated small hemorrhages increased the count, but the megakaryocytes were found at a low normal and Wright's figures were reduced. After administration of peptone, there was only a temporary fall in platelets, which returned to normal in twenty-four hours, and no change in megakaryocytes. Pyrodine failed to produce any change in the number of platelets or in that of megakaryocytes, and asphyxia, previously used by Bianchini and collaborators,<sup>30</sup> and supposed to produce both increase in circulating platelets and the appearance of numbers of megakaryocytes in the spleen of young kittens, failed in Petri's hands to do either. The gist of this portion of Petri's work is, first, his negative results after the use of some of the experimental agents previously reported on and second, and chiefly, the absence of any correlation between changes in the platelet level and in the megakaryocyte count.

Petri's blood platelet bleeding, while theoretically "ideal," presented certain insurmountable practical difficulties. Theoretically, the method should have succeeded in removing only the platelets from the blood without affecting the other elements, but an immediate, progressive and profound anemia always developed in the animals and they died after the sixth bleeding. Experiments on the reinjection of different parts of the blood, with and without citrate, convinced him that the fatal outcome was due to the reinjection of plasma, but the reason for this remained undetermined. "Ideal blood platelet bleeding" did produce great fluctuations in the platelet count, with a tendency to early fall and subsequent recovery; even this, however, was not constant, as one animal showed

29. Petri, S.: *Acta path. et microbiol. Scandinav.* **2**:23, 97, 277 and 357, 1925; **3**:432, 1926.

30. Bianchini, G., cited by Petri (footnote 29).

a primary rise. Megakaryocytes were either high, low or normal, and showed no correlation with the platelet count. He did, however, report a conspicuous increase in phenomena of degeneration in the megakaryocytes and the presence of leukocytes within their cytoplasm; these seemed sometimes to be phagocytosed by a healthy megakaryocyte, and sometimes to be invading a damaged megakaryocyte. Since blood platelet bleeding failed to produce a constant progressive change in either direction in the platelet count, no constant change in megakaryocyte count should have been anticipated. It is, moreover, unfortunate that Petri did not kill his animals when the maximum change in platelet number in any case had been obtained. For example, the megakaryocytes could hardly be expected to reflect changes in the number of platelets sixteen days after the last blood platelet bleeding and eleven days after the platelets had recovered, from a primary fall, to normal levels. Since, however, Petri alone, among all investigators of Wright's theory, reported in detail differential counts of megakaryocytes on all of his experimental animals, his results cannot be disregarded.

Petri gave additional reasons for his opposition to Wright's theory. First, he denied any similarity between the cytoplasm of megakaryocytes and platelets in fixed material, either in tissues or in coagulated blood in doubly ligated blood vessels, although he attempted to follow exactly Wright's technic for the preparation of sections. Second, he studied carefully in serial sections thirty megakaryocytes exhibiting Wright's figures, and always found the pseudopods attached to the main cytoplasmic mass. This proved, he maintained, that pseudopods are never broken away from the megakaryocyte to form platelets. Third, since he saw large, fully granulated megakaryocytes showing signs of degeneration—namely, pyknotic nuclei, chromatin inclusions and vacuoles—he reasoned that the megakaryocyte therefore passes through stages of degeneration normal to any cell and not a special type of degeneration in which the cytoplasm is broken up into platelets. Fourth, he considered "Felderung" to be due to chemical, toxic or postmortem degeneration, to obliquity of the section or, in smear preparations, to bursting of the cell and rubbing together of the granules.

Although there may be other grounds for objection to Wright's theory, these offered by Petri are scarcely valid. In the first place, the similarity between the cytoplasm of megakaryocytes and platelets in sections can be repeatedly demonstrated by proper technic. Secondly, formation of pseudopods is regarded as only a preliminary step to fragmentation, and although large masses of cytoplasm do appear in the blood stream under conditions of abnormally rapid formation of platelets, they are supposed usually to fragment without preliminary detachment. Third, the occurrence of (toxic?) degeneration in any form of parent bloodcell hardly proves that that cell might not, under

normal conditions, have passed through a different cycle to a different termination. "Felderung" remains a matter of opinion in the interpretation of undeniable, definite changes.

#### BONE MARROW WITH SPECIAL REGARD TO MEGAKARYOCYTES

It will be apparent from the above résumé of previous experimental work that any serious investigation concerned with Wright's theory must include not merely cursory observation, but very painstaking study of the bone marrow of the experimental animals. Ogata<sup>4</sup> long ago noted that the intensity of hematopoiesis varies greatly in animals of different ages, and that it is therefore necessary to use animals of the same age in any numerical study of the megakaryocytes. Sections must be selected from different levels in the marrow of the long bones, to determine whether or not hematopoiesis has been stimulated in areas of fatty marrow, where normally it is quiescent, and, as Ogata emphasized, in any comparison between different animals sections from the same level must be used.

The factor, however, that renders a numerical study of the megakaryocytes most difficult and most liable to fallacy is the complexity of forms assumed by this cell. First, there are the forms appearing during the course of normal development, maturation and functional activity. Wright<sup>2b</sup> pointed out the embryonic precursor of the megakaryocyte, and Ogata<sup>4</sup> and Downey<sup>31</sup> amplified his description of the megakaryocyte in functional activity, while Ferrata and Rinaldi<sup>18</sup> described the origin and earlier stages of development in detail. From the hemocytoblast (large lymphocyte of Maximow?) and the hemohistioblast (resting wandering cell?) in the embryo, but chiefly from the hemocytoblast in the adult, the "megakaryoblast," with intensely basophil cytoplasm free from granules, develops by simple hypertrophy. Coincident with increase in size of the cell, the nucleus divides mitotically or amitotically and a coarse net of chromatin is formed within it. The cell is now a "lymphoid megakaryocyte." Azurophil granules accumulate, at first in the perinuclear zone, later filling the entire cytoplasm. The cell is now, according to Ferrata and Rinaldi, a "mature megakaryocyte." The appearance of pseudopods, which, according to Wright, project into the lumina of sinusoids (Wright's figures), the grouping of granules into smaller and smaller masses as first described by Wright<sup>2b</sup> and later by Ogata<sup>4</sup> and Downey,<sup>31</sup> the fragmentation of pseudopods into platelets, either before or after the pseudopod has broken away from its parent cell, all indicate the maturity and functional activity of the megakaryocyte. The "naked nucleus" so often observed must obviously be interpreted as the end-result of loss of cytoplasm by the formation and

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31. Downey, H.: *Folia haemat.* **15**:25, 1913.



separation of pseudopods. In times of rapid regeneration of platelets, these processes may be telescoped—the formation of platelets from megakaryoblasts and the appearance in the blood stream of megakaryocytic pseudopods not yet showing “Felderung” have been mentioned.

Many authors have noted, in pathologic conditions, an increase in the number of either young or degenerating forms of the megakaryocyte, i. e., in forms which deviate from the classic description of the adult megakaryocyte given by Schridde<sup>32</sup> and by Wright.<sup>2</sup> Ferrata and Rinaldi<sup>18</sup> found that megakaryoblasts were increased in the marrow of their experimental animals. Bedson and Johnston<sup>23</sup> reported an increase in lymphoid and immature megakaryocytes after the use of antiplatelet serum. Bunting<sup>13b</sup> observed that young megakaryocytes with merely bilobed nuclei were numerous in marrows in which active regeneration of megakaryocytes was taking place, and that during rapid formation of platelets 50 per cent of these cells had little or no cytoplasm. Klaschen,<sup>33</sup> although he reported no platelet counts, studied the megakaryocytes in the spleen of the mouse after the injection of caseosan, and found (1) lymphoid, nongranular premegakaryocytes with large vesicular nuclei, (2) young, small megakaryocytes, round or irregular, with basophil cytoplasm and a minimum of granules, these cells being often arranged in groups as though syncytial, and (3) large, typical, mature megakaryocytes undergoing fragmentation. Firket<sup>34</sup> reported that when rabbits had a low platelet count (from 159,000 to 267,000) at the time of death, eight days after the last injection of saponin, the megakaryocytes, though numerous, were in the lymphoid stage, while in an animal that had received less saponin and had 474,000 platelets, 72.5 per cent of the megakaryocytes were granular. Dyke's observation that a high count of nongranular megakaryocytes may be present in purpura has been noted. Gáspár<sup>35</sup> made an observation that clarifies considerably the whole subject of the relation of platelets to megakaryocytes. He studied cases of pernicious anemia, aplastic anemia and purpura with low platelet counts, and found that the platelet number could be correlated only with the number of functional megakaryocytes and not with the total number. He classed as nonfunctional (1) young megakaryocytes with granules only in the central part of the cell and without pseudopods and (2) damaged megakaryocytes showing a pyknotic nucleus and loss of staining power of the granules, the nuclear preceeding the cytoplasmic damage.

This leads to the second factor that may render a numerical correlation between megakaryocytes and platelets more complex, namely, the

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32. Schridde, H., cited by Ogata (footnote 4).

33. Klaschen, L. V.: *Virchows Arch. f. path. Anat.* **237**:184, 1922.

34. Firket, J.: *Compt. rend. Soc. de biol.* **87**:84 and 86, 1922.

35. Gáspár, S.: *Frankfurt. Ztschr. f. Path.* **34**:460, 1926.

appearance of phenomena of degeneration in megakaryocytes. These phenomena have been repeatedly noted by many workers, and their occurrence is usually regarded as evidence of loss of function on the part of the megakaryocyte.

Third, no departure from the classic description of the megakaryocyte is more conspicuous and no phenomenon is more difficult to reconcile with Wright's theory than the inclusion of the other blood cells within the cytoplasm of the megakaryocyte. Three hypotheses in explanation of this are possible:

1. The included cells merely lie accidentally within lacunae in the cytoplasm of an irregularly shaped megakaryocyte; this seems most plausible when only one or a few cells are found at or near the periphery, especially when both megakaryocyte and included cells seem normal and a narrow clear space (attributed by Seeliger to shrinkage of both cytoplasm) appears around the included cell. The appearance of a complete ring of megakaryocyte cytoplasm around the blood cell might then be accounted for by the plane of section. This hypothesis, however, becomes untenable when the cytoplasm of the megakaryocyte is riddled with blood cells.

2. The megakaryocyte phagocytoses the blood cells. This theory seems most plausible if the megakaryocyte is normal, if the included cells are definitely damaged, and if erythrocytes, which are nonameboid, are included.

3. The megakaryocyte is being invaded by phagocytic leukocytes, a theory that is rendered almost necessary if the megakaryocyte shows pyknosis of its nucleus and disintegration of its cytoplasm while the included cells appear normal, and that is further strengthened if it can be shown that erythrocytes are never found in the depth of the cytoplasm.

Woodcock<sup>36</sup> believed that the megakaryocytes phagocytose and digest erythrocytes and manufacture platelets out of the pabulum. Seeliger<sup>11</sup> found erythrocytes, leukocytes and lymphocytes included within the cytoplasm of mature megakaryocytes showing "Felderung" at the periphery; the signs of degeneration and digestion appearing in the included cells, which he described in detail, convinced him that it was actually phagocytosis on the part of the megakaryocyte. Petri's observation of the same phenomenon has been mentioned; the inclusions consisted of granulocytes, erythrocytes and "homogeneous platelets." Some observations of my own on the subject of the inclusion of blood cells within the cytoplasm of megakaryocytes will be given later. Phagocytosis of bacteria by megakaryocytes was reported by Katzenstein.<sup>37</sup>

The foregoing detailed descriptions of the megakaryocyte have been reviewed at such length because of its outstanding importance in the interpretation of results in experiments of this nature. Wright's theory rests fundamentally on the demonstration of a correlation between changes in the number of circulating blood platelets and changes in the

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36. Woodcock, cited by Bedson (footnote 22).

37. Katzenstein, W. F.: *Ztschr. f. d. ges. exper. Med.* 48:607, 1926.

number, or in the evidence of platelet-forming activity, of megakaryocytes. In order to determine when a megakaryocyte shows evidence of platelet-forming activity, all the other phenomena exhibited by megakaryocytes must be properly interpreted. For example, is the megakaryocyte phagocytic? Or do leukocytes remove damaged megakaryocytes? Is there an increase in damaged megakaryocytes during or following an increase in platelets? Does the presence of a pyknotic nucleus signify that platelet-forming activity has been or will soon be arrested? If phagocytosis is an accessory function of the megakaryocyte, what are the conditions that cause it to assume this function? Can a megakaryocyte phagocytose blood cells or other material and at the same time form platelets? Or does phagocytosis, assuming that it occurs, interfere with the platelet-forming function? Until such questions can be satisfactorily answered, that exact correlation between platelets and megakaryocytes on which Wright's theory depends cannot be established beyond doubt.

With regard to the study of numerical changes in megakaryocytes, only Bunting,<sup>13b</sup> Ogata,<sup>4</sup> Firket<sup>34</sup> and Petri<sup>29</sup> reported actual counts of megakaryocytes on some or all of their animals, and only Petri included differential counts, although other writers stated that such enumerations had been made. The failure to report both total and differential counts of the megakaryocytes constitutes a serious omission from any study concerned with Wright's theory.

#### IDEAL APPROACH TO PROBLEM OF ORIGIN OF PLATELETS

Experimental work that purposes to deal with the problem of the origin of platelets should therefore avoid certain errors inherent in much of the older work and should attempt to fulfil certain ideal requirements. The first requisite is a proper experimental agent. Practically all of the agents thus far mentioned have augmented the production of platelets only secondarily; i. e., as a compensation to the primary removal or destruction produced by the experimental method or agent. This mode of action is not in itself objectionable, but chemical agents acting in this way are invariably more or less toxic. Toxicity likewise need not be objectionable since the dosage of toxic substances, at least theoretically, could be graded to produce a stimulating effect with a primary increase in the number of platelets. Toxic agents, however, have acted on all the blood elements, thus complicating the picture of the bone marrow by affecting all types of hematopoiesis. The same objection applies to bleeding as an experimental method. In larger doses the toxic agents have damaged or destroyed megakaryocytes, as well as platelets, and have even produced generalized destruction of the bone marrow. It is clear that a damaged bone marrow cannot react in a normal fashion.

The ideal agent, therefore, should produce an isolated effect on platelets, preferably a primary rather than a compensatory increase, and should be relatively nontoxic. Petri, by means of his ideal blood platelet bleeding and Bedson by his antiplatelet serum, attempted, but failed, to devise an agent acting on blood platelets alone. Blood platelet bleeding produced profound anemia in a very short time, and the antiplatelet serum, though carefully absorbed out with other antigens, was likewise attended by an untoward effect (not due to agglutination) on the red cells.

As a second requirement for adequately controlled experimental work on the origin of platelets, the studies of tissues must take cognizance not merely of numerical changes in the megakaryocytes, but of the character of these cells, whether young, mature or defunct, and of the occurrence of degenerative phenomena and cell inclusions. Third, the tissues must be taken for study at different stages during the rise and fall of the number of platelets to ascertain whether there is any correlated change in functional megakaryocytes. Fourth, the animals should be approximately of the same age and the sections for comparative studies taken from the same levels of the bone marrow. Finally, certain precautions, which will be described in part II under the heading "Material and Methods," must be observed in the technic of counting platelets and in the handling of tissues.

In the experiments that form the basis of parts II and III of this paper two different methods have been used in attempts to increase the blood platelets selectively, namely, irradiation with ultraviolet light and infection with a specific bacterium. The use of certain operative procedures, including splenectomy, will be reported in a later publication. None of these meets all of the requirements for an "ideal agent," but each is sufficiently selective to justify its use in experiments of this kind. The nature of the action and the degree of selectivity in each case will be discussed subsequently.

## II. EXPERIMENTS WITH ULTRAVIOLET LIGHT

As was stated in part I, experiments were undertaken which had for their purpose the induction of thrombocytosis and the study of the correlated changes in the bone marrow. It was hoped that these studies might contribute to the solution of the problem of the origin of blood platelets.

In the first group of experiments ultraviolet light was used to induce thrombocytosis. The fact that ultraviolet light has such an influence on the level of platelets had been previously observed. Cramer and Drew<sup>17</sup> showed that thrombopenia developing when rats were reared in darkness on a diet deficient in vitamin A could be overcome by ex-

posure to ultraviolet light. Laurens and Sooy<sup>38</sup> (1924) were able to produce an increase in blood platelets in the rat by use of the same agent. Sooy and Moise<sup>39</sup> (1926) treated patients for purpura hemorrhagica with the quartz lamp, and found that the increased number of platelets so obtained was accompanied by clinical improvement. Sanford<sup>40</sup> (1927) found that, besides inducing other changes in the blood, ultraviolet light raises the platelet count in new-born infants. Preliminary experiments on the effect of ultraviolet light on the blood platelets of rabbits have been reported by myself<sup>41</sup> (1926), and similar experiments in greater detail have been reported by Hardy<sup>42</sup> (1927) and by Vannfalt<sup>43</sup> (1929). Similar results were obtained with dogs by Miles and Laurens<sup>44</sup> (1926) and Mayerson and Laurens<sup>45</sup> (1928); irradiation by means of the carbon arc was used in their experiments.

#### MATERIAL AND METHODS

Two groups of rabbits were used for the experiments, seven normal animals and seven subjected to irradiation from the mercury vapor quartz lamp. Daily blood counts were made on each animal throughout the course of an experiment, and an additional count was made on the last day just before the animal was killed. Blood samples for the counts were taken from the marginal vein of the ear in every case. Erythrocyte and leukocyte counts were made in the usual manner, the same pipets being used for all counts. Enumeration of blood platelets was accomplished by an indirect method similar to that of Fonio.<sup>46</sup> The ratio of platelets to erythrocytes was determined by counts made on dried, stained films and the number of platelets calculated from the previously determined erythrocyte count. The films were made from a freely flowing drop of blood obtained by puncturing a vein of the ear through a drop of isotonic anticoagulant fluid. The method has been described in detail (Gunn,<sup>41</sup> Gunn and Vaughan<sup>47</sup>). This method has been criticized by some authors, but I have found it to yield more uniform results than any other method employed, and it continues after long use to be satisfactory.

The animals were killed by a blow on the back of the head. Bone marrow and other tissues were removed with dispatch and immediately fixed while still warm in Zenker's fluid plus solution of formaldehyde and Müller's solution plus

38. Laurens, H., and Sooy, J. W.: *Proc. Soc. Exper. Biol. & Med.* **22**:114, 1924.

39. Sooy, J. W., and Moise, T. S.: *Treatment of Idiopathic Purpura Hemorrhagica by Exposure to Mercury Vapor Quartz Lamp; Preliminary Report*, *J. A. M. A.* **87**:94, 1926.

40. Sanford, H. N.: *Effect of Ultraviolet Light on the Blood of New-Born Infants*, *Am. J. Dis. Child.* **33**:50, 1927.

41. Gunn, F. D.: *Proc. Soc. Exper. Biol. & Med.* **24**:120, 1926.

42. Hardy, M.: *Am. J. Hyg.* **7**:811, 1927.

43. Vannfalt, K. A.: *Compt. rend. Soc. d. biol.* **101**:607, 1929.

44. Miles, A. L., and Laurens, H.: *Am. J. Physiol.* **75**:462, 1926.

45. Mayerson, H. S., and Laurens, H.: *Am. J. Physiol.* **86**:1, 1928.

46. Fonio, A.: *Cor.-Bl. f. schweiz. Aerzte* **45**:1505, 1915.

47. Gunn, F. D., and Vaughan, S. L.: *Anat. Rec.* **45**:59, 1930.

solution of formaldehyde. Disks about 3 mm. in thickness were cut with a jeweler's saw from the tibia and femur at different levels—(1) just below the trochanter, (2) through the middle, (3) just above the epicondyles of the femur and (4) from the upper end of the tibia. The lower part of the tibia was then cracked open and the marrow examined to ascertain the level of the line of demarcation between red and fatty marrow. Normally, in animals of the size and age used, this line is located at about the middle of the tibia; the marrow of the femur is red throughout. When the marrow is actively hyperplastic, the red marrow extends farther distally in the tibia, sometimes throughout its whole length.

The tissues in Zenker's fluid plus solution of formaldehyde were allowed to fix for from six to seven hours; those in Müller's solution plus solution of formaldehyde for from twenty to twenty-four hours. At this time the marrow was removed from the bony rings after first being loosened from the bone with a thin pointed blade. All tissues were washed in running water for twenty-four hours. To prevent shrinkage of the tissues the following precautions were observed: Dehydration in the higher alcohols was permitted only for minimum periods; chloroform was used for clearing; the period of infiltration in pure paraffin at 56 C. was two hours.

Sections were cut at 5 microns. Two principal stains were used, Giemsa's on the material fixed in Zenker's fluid and Wright's tissue stain, prepared as described by him, on the material fixed in Müller's solution. Giemsa's stain does not stain the platelets clearly in sections and does not give a brilliant stain on the granules of the megakaryocytes, but is more satisfactory than Wright's for studying the nuclear changes, and is the method of choice for all other myeloid elements. Wright's stain gives unsatisfactory results on the material fixed in Zenker's fluid.

Studies of bone marrow included (1) general observations on hematopoiesis and (2) numerical and differential counts on megakaryocytes. The counts were made with a Spencer binocular microscope, 10 $\times$  oculars and the 4 mm. objective being used. With this system of lenses each visual field represents an area measuring 0.35 mm. in diameter. Every megakaryocyte showing a portion of the nucleus was counted in 400 visual fields, 100 fields from each of the four levels mentioned.

Megakaryocytes were divided into (1) mature, (2) lymphoid and (3) degenerating forms. "Mature cells" comprised those conforming to the classic description and also the young megakaryocytes with granules appearing only in the perinuclear zone. "Lymphoid megakaryocytes" included those of any size with basophil cytoplasm devoid of granules; the smallest of these had a pale blue vesicular nucleus and a narrow rim of deeply basophil cytoplasm; the larger had a complex nucleus similar to that in the mature form. Degeneration was evidenced by pyknosis of the nucleus, by loss of distinctness and brilliancy in the granules of the cytoplasm, and by a tendency of the cytoplasm to take the acid stain. "Naked nuclei" were classed with degenerating cells, since practically all of them showed distinct evidence of degeneration in the forms of chromatolysis and pyknosis. "Naked nuclei" in a few instances constituted a large proportion of the cells classed as degenerating. The sum of these three types gave the total number of megakaryocytes. In addition, separate enumerations were made (1) of megakaryocytes in the cytoplasm of which leukocytes were seen and (2) of Wright's figures. The included leukocytes were almost invariably polymorphonuclear and rarely were lymphoid; erythrocytes were never found included. Although sometimes the leukocytes were degenerating, more commonly the evidences of degeneration appeared in the megakaryocyte. The inclusion of leuko-

cytes occurred, then, usually in degenerating megakaryocytes, but sometimes in apparently normal mature cells and very rarely in lymphoid forms. Wright's figures included those cells showing "Perlenketten," pseudopods with clumping of granules, or fragmentation of the entire cytoplasm.

The source of the ultraviolet light used in these experiments was the mercury vapor quartz lamp (Victor 110 volts, 10 amperes). After normal counts were established, the back of the animal was shaved or closely clipped and the animal exposed daily to increasing doses, beginning with an exposure of about five minutes at a distance of 16 inches (40.7 cm.) and increasing to as much as from twenty to thirty minutes at the same distance over a period of from five to fourteen days. Seventeen animals were treated in this way, all but two or three of which responded with a considerable increase in platelets. For the present study seven animals were selected which showed an increase of from 37.7 to 179.5 per cent above the average normal, with an average increase of 96.2 per cent. Other animals showed increases as great or greater than these, but were allowed to survive, so that the duration of such increases could be ascertained. Others had been subjected to experimental conditions other than that of irradiation by ultraviolet light and so were not suitable for this study. Of the seven selected, two were killed while the platelets were at a high level and still increasing, two on the second day after the maximum count and while the platelets were declining, one on the third and two on the fourth day.

As controls, seven normal animals were used and were subjected to no manipulation other than that incident to daily complete blood counts. In each case an additional blood count was made on the fourth day just before the animal was killed.

All the animals were kept in cages in a moderately well lighted room, but out of direct sunlight, both before and during the experiments, and were fed on a diet of oats, carrots and alfalfa hay.

## RESULTS

The results have been tabulated in tables 1, 2 and 3.

*Normal Animals.*—The results of the enumeration of blood elements and of the differential count of megakaryocytes in the normal animal were surprisingly constant (table 1). Only the final count of the erythrocytes is recorded in the table; the average erythrocyte count was 6,346,000. The average leukocyte count was 9,692, with variations between 6,300 and 12,200. Platelet counts varied between 393,000 and 763,000 (the lowest and the highest single counts), with an average normal count of 626,000. The average normal platelet count, including those on the experimental animals before treatment was 750,000. The count on rabbit 54 was not included in this average because this animal showed a spontaneous high normal level of unexplained origin.

The total megakaryocyte count averaged 239.7 per hundred visual fields, distributed among the three types as follows: mature, 169.3; lymphoid, 47, and degenerating, 23.5. Of these, 4.4 (per hundred visual fields) showed "phagocytosis" and 4.1 showed Wright's figures. The total megakaryocyte count varied between 204.3 and 312.5; the mature between 137 and 223; the lymphoid, between 31.5 and 65.5, and

TABLE 1.—*Normal Animals*

Rabbit	Weight, Gm.	Red Blood Cells at Autopsy, Thous.	White Blood Cells at Autopsy	Platelets in Thousands			Megakaryocytes, Average per Hundred Visual Fields					Condition of Bone Marrow		
				Low	High	Average	Level at Autopsy	Total	Mature	Lymphoid	Degenerating		Phagocyte	Wright's Figures
16.....	1,330	5,580	.....	...	...	955	955	201.3	137.0	41.8	25.5	2.8	2.5	Normal
66.....	1,500	6,520	6,300	502	580	544	502	236.9	168.8	46.8	21.3	3.8	5.0	Normal
67.....	1,555	6,780	8,700	539	738	630	638	226.0	158.0	42.0	26.0	3.5	5.5	Normal
68.....	1,635	6,600	9,100	393	601	524	561	254.0	172.5	61.5	17.0	5.5	2.5	Normal
69.....	1,905	5,850	11,000	415	588	483	479	312.5	223.0	65.5	24.0	4.0	6.5	Normal
70.....	1,470	6,150	12,200	537	763	676	763	231.0	167.5	37.0	26.5	8.5	3.5	Normal
71.....	1,680	6,940	10,850	527	603	567	576	213.5	158.0	31.5	24.0	2.5	3.5	Normal
Average.....	1,654	6,346	9,692	489	636	626	612	239.7	169.3	47.0	23.5	4.4	4.1	

TABLE 2.—*Animals Treated with Ultraviolet Light*

Rab- bit*	Weight, Gm.	Red Cells, Thous- ands	White Blood Cells	Platelets in Thousands						Days from Peak to Autopsy	Megakaryocytes, Average per Hundred Visual Fields					Hyperplasia of Bone Marrow	
				Nor- mal Low	Nor- mal High	Nor- mal Average	Experi- mental Maxi- mum	Level at Autopsy	Increase, per Cent		Total	Mature	Lym- phoid	Degen- erating	Phngo- cytic		Wright's Figures
13	1,275	4,715	10,200	714	1,248	942	1,886	1,734	100.2	0	222.0	167.5	24.5	30.0	17.5	6.3	Slight to moderate
17	1,500	5,360	.....	521	794	643	1,467	1,120	128.1	2	317.3	235.0	35.8	40.5	16.0	9.3	Moderate
20	2,179	4,920	5,200	926	959	943	2,636	1,588	179.5	4	404.5	318.5	49.0	37.0	9.5	12.0	Moderate
46	1,710	6,510	22,100	688	828	760	2,000	1,622	162.2	3	259.3	114.0	7.5	137.8	137.3	8.5	Moderate
48	1,918	6,680	12,650	636	872	766	1,323	1,323	72.7	0	204.5	164.3	17.0	23.2	31.5	11.8	(Normal)
49	2,915	6,450	16,350	793	873	828	1,316	942	59.0	4	218.1	165.3	30.8	22.0	21.5	5.3	(Normal)
54	1,255	5,450	12,000	1,242	2,066	1,529	2,105	1,510	37.7	2	342.0	274.5	30.0	38.0	10.0	12.5	(Normal)
Aver.	1,822	5,629	11,280				1,789	1,370	96.2		281.7	220.8	31.2	32.8	17.7	9.5	

\* No. 46 omitted from all averages in the table for reasons given in the text.



the degenerating between, 17 and 26.5. The highest number of phagocytic forms was 8.5; the lowest, 2.5. The highest number of Wright's figures was 6.5; the lowest, 2.5.

*Animals Treated with Ultraviolet Light.*—Ultraviolet light produced increases in the count of circulating blood platelets varying from 37.7 per cent to 179.5 per cent, or an average increase of 96.2 per cent. The highest level attained under the influence of ultraviolet light was 2,636,000; the average of the highest levels was 1,789,000, and the average level just before autopsy was 1,370,000. The animal showing the smallest rise in platelets was no. 54, which had had a spontaneous rise before treatment and, therefore, the highest average normal level.

Megakaryocytes showed an average total of 284.7 per hundred visual fields, which was an increase of 19 per cent above the normal average total, although the total in four of the seven animals fell within normal limits. The total was distributed as follows: mature, 220.8; lymphoid, 31.2; degenerating, 32.8, or an increase in mature and degenerating forms and a decrease in lymphoid forms. The most marked variation from the normal picture, however, was found in the occurrence of 17.7 phagocytic forms, a considerable increase, and of 9.5 Wright's figures, which was more than double the normal number. Animal 46 was omitted from the calculation of all averages in this section because its bone marrow was distinctly different from that of the other animals treated with ultraviolet light and strikingly similar to that of infected animals, which will be described in part 3. Its leukocyte count of 22,100 at autopsy supported the suspicion that the animal was infected.

Distinct evidence of general hyperplasia of the bone marrow was present in four of the seven rabbits of this group. The other three appeared normal, both grossly and microscopically. In only one (20) was there gross evidence of hyperplasia, and in this one the red marrow extended nearly to the lower end of the tibia. In the others, the line of demarcation between red and fatty marrow was at about the middle or slightly below the middle of the tibia. Microscopically, a moderate degree of hyperplasia involving both erythropoietic and granulopoietic was found in three animals (17, 20 and 46) and a somewhat milder degree in a fourth (13). It will be observed from table 2 that these four animals showed the greatest platelet response.

#### SUMMARY AND COMMENT

The experiments showed that under the influence of ultraviolet light an elevation of the level of the blood platelets occurred that was strikingly correlated with changes in the number and the form of the megakaryocytes in the bone marrow. Certain definite tendencies were apparent in the megakaryocytes. First, they showed, if average figures

are considered, an increase in total number, although individual cases fell within the limits of normal variation, which are wide. Second, Wright's figures were distinctly increased. Although two animals failed to show increases in Wright's figures above the highest normal, the number in both was greater than the average normal; rabbit 13 merely reached the high normal; rabbit 49 had had only a 59 per cent increase in platelets and was not killed until four days after the peak; at this time the platelet level was nearly normal (942,000). Third, there was a noticeable diminution in lymphoid forms and a tendency to maturation; this was manifested by a decrease in lymphoid forms of 33.6 per cent, by an increase in mature forms of 30.4 per cent, and by an increase in degenerating forms of 39.5 per cent. These are the average figures for the entire group.

If the data obtained from the seven experimental animals are analyzed more closely, arranging the animals according to the number of days that elapsed between the time of the maximum platelet count and

TABLE 3.—*Animals Treated with Ultraviolet Light*

Days from Platelet Peak to Autopsy	Rab- bits	Platelets		Percentage of Increase or Decrease in Total and Differential Counts of Megakaryocytes					
		Nor- mal Aver- age	Increase at Autopsy, per Cent	Total	Lym- phoid	Mature	Degen- erating	Phago- cytic	Wright's Figures
At peak.....	2	854	78.4	-12.8	-56.0	-2.0	+13.2	+457.0	+121.1
2d day after.....	2	1,086	36.5	+37.5	-30.0	+50.4	+79.8	+195.5	+165.9
3d day after.....	1	760	34.5	+8.2	-81.9	-32.7	+486.4	+3,020.5	+107.3
4th day after....	2	885	41.1	+29.9	-15.1	+42.9	+25.1	+252.3	+111.0

the time of autopsy, certain exceptions to these general trends appear (table 3). First, the average total number of megakaryocytes in the two animals killed at the peak is actually less (12.8 per cent) than the normal average total and the increase in the total number in the animal killed on the third day is but 8.2 per cent. These figures, however, all fall within the limits of normal variation, which, as previously stated, are wide. Second, the same three animals failed to show an increase in the number of mature megakaryocytes. In other words, an exact correlation between the number of megakaryocytes and the level of the platelets at the time of autopsy cannot be demonstrated, just as Petri maintained and contrary to the claims of most of the protagonists of Wright's theory. This fact serves merely to emphasize the need for most careful analysis of the megakaryocytes. When such an analysis is made, it is seen that none of the animals failed to illustrate the tendency to a shift from lymphoid to mature and degenerating forms—in other words, an increased rate of maturation. The evidence from this study of thrombocytosis induced by ultraviolet light and from a study of the correlated changes in the bone marrow tends, I believe, to substantiate Wright's theory.

A possible explanation for the striking increase in the phagocytic forms became apparent only when the results obtained by the use of ultraviolet light were compared with those obtained by the use of other agents. At the same time, a theory of the mode of action of ultraviolet light on megakaryocytes and platelets was suggested. These theories will be discussed in part 3.

### III. EXPERIMENTS WITH A SPECIFIC BACTERIAL INFECTION

The experimental induction of thrombocytosis and the study of correlated changes in the bone marrow were continued in a second group of animals infected with a specific organism, hereafter referred to as *Bacillus thrombocyto-genes*. The early results of these studies have been reported in a preliminary paper.<sup>48</sup>

The influence of bacterial infections on the platelets and the significance of alterations in the number of platelets in infections is rather obscure, in spite of the fact that a large number of observations have been made. These observations have been almost entirely confined to clinical cases. One of the most intensive studies was made by Reimann,<sup>49</sup> in pneumococcal infections. He found that in lobar pneumonia, thrombopenia occurred during the febrile period of the disease and lasted until the temperature began to fall by crisis or by lysis, after which the platelets began to increase in number, reaching a level considerably above normal and finally subsiding gradually to a normal level in about two weeks. This has been found to be true, in general, of a number of other acute infections. Most observers have found the platelets decreasing in number at the onset of the fever, increasing gradually during the disease, rising above the normal level after the temperature has subsided, remaining high for one or two weeks and subsiding gradually to normal. As exceptions to this general rule, scarlet fever has been found to show no thrombopenia during the febrile stage, but rather an increase in platelets (Stahl<sup>50</sup>). Bannerman<sup>51</sup> observed an increase in platelets in the common cold. Platelet counts in tuberculosis indicate an inverse ratio between thrombocytosis and the patient's resistance to the disease. Bannerman<sup>52</sup> found that patients with a count above 510,000 usually showed evidence of a rapidly down-grade course, while counts below 400,000 indicated a good prognosis.

The response of blood platelets in rabbits to infection with *B. thrombocyto-genes* seems to be exceptional, as the thrombocytosis appears at the beginning of the infection without a preliminary thrombopenia.

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## MATERIAL AND METHODS

The rabbit from which *B. thrombocytoenes* was originally recovered had been subjected to splenectomy and had shown a resultant rise in platelets, which in a few days returned to the normal level. The animal was then given a course of treatments with ultraviolet light. The number of platelets again increased and was again decreasing after the treatment had been discontinued when, without apparent cause, a secondary rise occurred, which continued for thirteen days, reaching a maximum of 3,408,000 per cubic millimeter. The animal was killed

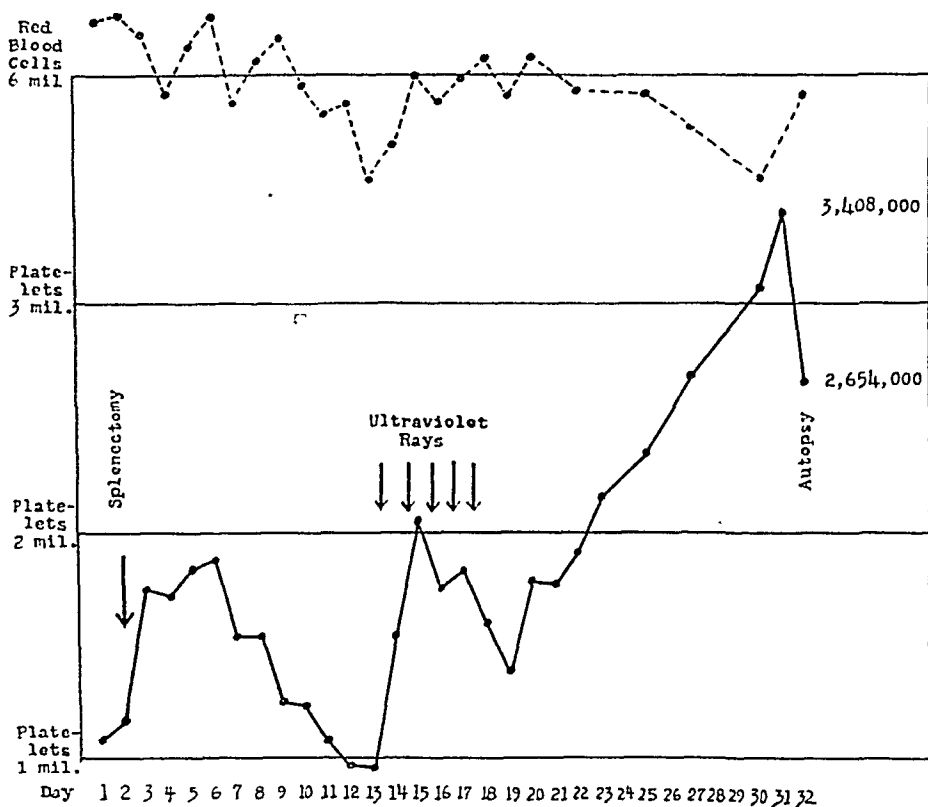


Chart 1.—The curve of the red blood cell count (dotted line) and of the platelet count (solid line) of rabbit 21, an albino male, infected with *Bacillus thrombocytoenes*.

one day after the maximum count was obtained, when the platelet count had dropped to 2,654,000 (fig. 1). The only striking pathologic change found was a rather extensive cheesy mass infiltrating the lateral abdominal wall near the site of operation, and from this the bacillus was obtained in pure culture. The only other abnormality found was a hyperplastic bone marrow. Reinjection of the subcultures of the bacillus was effective, shortly after its original isolation, in producing a striking increase of platelets in the other four rabbits included in this group.

Figure 2 illustrates the platelet response in one of these animals. A number of other rabbits similarly treated were allowed to recover. None of the animals exhibited as great a rise in platelets as that observed in the first, possibly because in the latter it was a summation of effects of the several agents used. The infection was attended by leukocytosis and in the large doses used was fatal in three animals. After nine months in subcultures the bacillus had lost much of its virulence for rabbits and its ability to affect the number of platelets was somewhat diminished.

The cultural and biologic characteristics of *B. thrombocytopenes* will be described in detail in a separate report. It grows on artificial

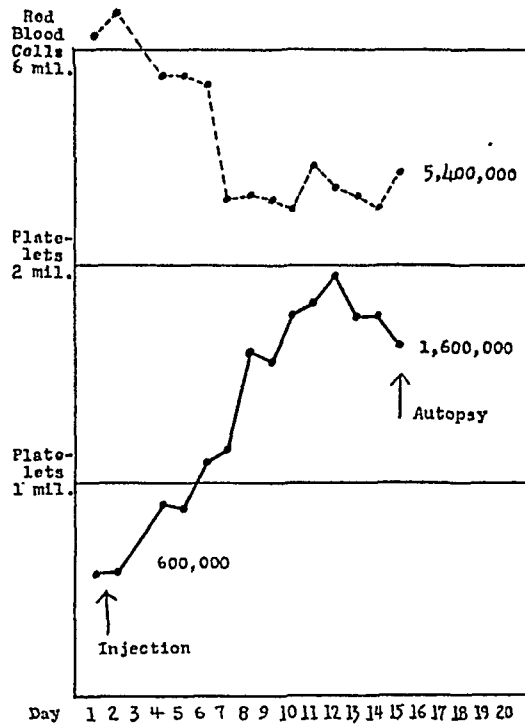


Chart 2.—The curve of the red blood cell count (dotted line) and of the platelet count (solid line) of rabbit 27, a black male, inoculated with *Bacillus thrombocytopenes*.

mediums as long, slender rods, forming long chains in bouillon and sometimes forming oval, subterminal spores. Reduced oxygen tension favors its growth. In old cultures the bacilli are positive to Gram's stain, but usually they are gram-negative.

In these experiments, forty-eight hour cultures on blood agar slants were suspended in sterile saline solution and injected subcutaneously and intramuscularly into each animal, except one. In this case an intravenous injection was made, but since it was found to be less effective than the subcutaneous inoculation it was discontinued. Each animal received the growth from one blood agar slant suspended in 1 cc. of saline solution.

TABLE 4.—*Animals Infected with the Bacillus Thrombocytoenes*

Rab-Weight, bit	Gm.	Thou- sands, at	White Blood Cells at Autopsy	Platelets in Thousands					Days from Peak to Autopsy	Megakaryocytes, Average per Hundred Visual Fields				Hyperplasia of Bone Marrow		
				Nor- mal Low	Nor- mal High	Nor- mal Average	Experi- mental Max- imum	Level Increase, at Autopsy		Total	Mature	Lym- phoid	Degen- erating		Phago- cyte	Wright's Figures
21	2,250	5,930	18,800	650	1,197	911	3,408	2,654	274.1	1	241.0	95.0	3.0	143.0	50.0	20.0
22	1,644	5,380	8,450	881	1,035	936	1,757	1,727	87.7	1	352.0	211.0	39.5	101.5	13.0	19.0
24	1,800	6,250	.....	688	688	.....	1,739	1,250	152.8	1	406.0	202.5	66.0	137.5	42.5	12.0
26	2,377	5,580	12,850	1,102	1,122	1,112	1,080	1,423	51.1	3	204.5	0.5	1.0	203.5	141.0	7.5
27	2,349	5,440	10,700	577	593	585	1,065	1,637	235.9	3	463.5	290.0	61.0	112.5	23.5	15.5
Aver.	2,084	5,716	12,700				2,110	1,738	160.3		333.4	159.8	34.1	139.6	54.6	14.8
Aver.*	1,654	6,346	9,692	489	636	626	.....	612	.....	..	239.7	169.3	47.0	23.5	4.4	4.1

\* Averages of normal animals for comparison.

Erythrocyte and platelet counts were made daily with frequent total and occasional differential leukocyte counts for each animal. The handling of tissues and the technic of enumerating megakaryocytes in sections of bone marrow were described in part 2.

#### RESULTS

This infection produced the highest levels of platelets obtained in any of the experiments. The highest number of platelets attained was 3,408,000, or an increase of 274.1 per cent; the lowest, in rabbit no. 26, was 1,680,000, or an increase of 51.1 per cent; the average increase for the group was 160.3 per cent. Megakaryocytes showed an average total of 333.4 per hundred visual fields, or an increase of 39 per cent above the normal average total, although in two of five animals the totals fell within normal limits. The distribution among the three types was extremely variable as reference to table 4 will show, but with a tendency to reduction in the number of lymphoid forms and a striking increase in the number of degenerating forms. There was also a marked increase in "phagocytic" forms, namely, to 39.6 (from an average normal of 4.4) and in Wright's figures to 14.8 (from 4.1). Reference to the table will show that rabbit 26 varied markedly from the others in this group. The animal showed at autopsy a pneumococcal pleuritis and pericarditis complicating the usual pathologic picture found in this infection. The significance of this mixed infection as a possible cause of the unusual picture of the bone marrow will be discussed.

The general picture of the bone marrow in all five of these animals was that of a high grade of myeloid activity. Grossly in all cases the red marrow extended downward in the tibia entirely or nearly to its lower end, and in two cases the marrow was softer and more friable than normal (26 and 27). Microscopic sections showed a great reduction in the size and number of the fat vacuoles. Myelopoiesis was active, pseudo-eosinophil myelocytes and metamyelocytes predominating.

#### SUMMARY AND COMMENT

The experiments in which *B. thrombocytogenes* was used as the agent to induce thrombocytosis showed the same results as were obtained from the use of ultraviolet light, but more striking. First, the total number of megakaryocytes reached higher levels than any previously obtained. Although in this group, as in the ultraviolet group, the total number of megakaryocytes in two animals lay within normal limits, the average increase was 39.1 per cent, as compared with 18.8 per cent in the ultraviolet group. Second, the number of Wright's figures was invariably higher than normal and showed an average increase of 261 per cent, as compared with one of 132 per cent in the ultraviolet group. Third, there was a most striking and peculiar change in the form of

the megakaryocytes. The number of lymphoid forms showed a decrease of 27.5 per cent, as compared with a decrease of 33.6 per cent in the ultraviolet group; the number of mature forms showed a decrease of 5.6 per cent, as compared with an increase of 30.4 per cent, while the number of degenerating forms received a tremendous augmentation of 494 per cent and that of phagocytic forms, of 1,141 per cent, as compared with 39.6 per cent and 302 per cent, respectively. The shift in forms was not, therefore, to mature forms, but beyond them, in the megakaryocyte cycle, to degenerating forms; there was, also, a conspicuous increase in the phenomenon of phagocytosis.

Two other significant changes in the form of megakaryocytes were observed. The first of these was an increase in the number of "naked nuclei." In the ultraviolet group of animals separate counts of naked nuclei were not made; these were included with degenerating megakaryocytes, because they practically always showed pyknosis or chromatolysis. It became apparent, however, that in the marrows in which degenerative forms were most abundant,\* "naked nuclei" were so numerous as to deserve special enumeration. Accordingly, they were counted differentially in two animals; rabbit 21 had 67 and rabbit 26 had 99 per hundred visual fields. Careful scrutiny revealed that many of the apparently "naked nuclei" still showed evidence of active formation of platelets in the form of a very narrow rim of cytoplasm from which small processes or occasionally long, finger-like, branching pseudopods with grouped granules ("Perlenketten") extended out and were almost hidden among the adjacent cells. The other phenomenon was the occurrence of numerous fragments of megakaryocytic cytoplasm, ranging in size down to that of a platelet. This was much more noticeable in animals of the infected group than in those of the ultraviolet light group, especially in rabbits 21 and 26, in which degenerating forms of megakaryocytes also were most numerous.

Other authors have suggested that "naked nuclei" or "nearly naked nuclei" and fragmenting cytoplasm are just as clear evidence of platelet-forming activity on the part of the megakaryocytes as are Wright's figures. When marked degeneration of megakaryocytes occurs in conjunction with these two features, the three together may be regarded, I believe, as evidence of recent overactivity and present exhaustion of the megakaryocytes.

The subject of "phagocytosis," so much discussed, deserves special mention. Three theories to explain the inclusion of other cells in the cytoplasm of the megakaryocytes have been enumerated, namely, that of accidental inclusion, that of true phagocytosis and that of invasion. The possibility of accidental inclusion of a few cells of any type cannot be excluded. In the marrows of my experimental animals of both



groups megakaryocytes with included cells invariably showed degenerative changes of the nucleus or of the cytoplasm or of both, while the leukocytes were apparently normal. This strongly suggests that so-called "phagocytosis" is really the invasion of damaged megakaryocytes by leukocytes (granulocytes), especially since the phenomenon occurred most abundantly in animals of the group infected with *B. thrombocytopogenes*, in which degenerating forms were most numerous and in which damage to the marrow was evident.

The probable mode of action of both experimental agents in causing thrombocytosis may now be suggested. The changes in form of the megakaryocytes observed in both groups of animals were similar in character; the difference was merely one of degree. Both agents apparently accelerated maturation of the megakaryocyte, increasing its platelet-forming activity and ultimately damaging the megakaryocyte. These facts suggest that both agents acted as irritative, probably toxic, stimuli to the megakaryocytes. It seems probable, also, that the introduction of a still more toxic agent, as occurred in rabbit 26 when it contracted an accidental infection with pneumococcus, can hasten degeneration to the point of actual inhibition of platelet-forming activity. This animal showed, it will be recalled, an increase of only 51.1 per cent in platelets, and in the marrow practically no lymphoid or mature megakaryocytes, but the greatest number of degenerating and "phagocytic" forms of any of the experimental animals and a relatively slight increase in Wright's figures. It will be recalled at this point that Duke<sup>19</sup> was able to produce stimulation of marrow and increase in platelets with small doses of toxic agents, and damage to megakaryocytes and decrease in platelets with large doses. It may seem remote to assume a similarity between the action on megakaryocytes of ultraviolet light and that of a toxin, whether introduced from without (diphtheria toxin, benzol) or produced within by *B. thrombocytopogenes*. The only explanation at present possible is to postulate a toxic substance arising from damage to tissues as has been suggested by Dawbarn, Earlam and Evans<sup>53</sup> and other workers.

The more marked changes in the megakaryocytes and the coincident greater rise in platelets shown throughout by the infected group indicate that this agent is more effective than ultraviolet light. It was also observed in the larger series of animals treated with ultraviolet light, that the effect of this agent in raising the level of the platelets could not be prolonged beyond six or seven days. It is possible that the thickening of the skin following the inflammatory reaction to ultra-

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53. Dawbarn, R. Y.; Earlam, F., and Evans, W. H.: J. Path. & Bact. **31**:833, 1928.

violet light may prevent penetration of the rays and so limit the effect of irradiation. Infection, on the other hand, is progressive; it continues to stimulate the formation of platelets either until the animal recovers from the infection (as shown by animals not included in the present series) or until the megakaryocytes are exhausted.

The hyperplasia of bone marrow occurring in these rabbits deserves discussion. The degree of general hyperplasia was correlated to a certain extent with the intensity and duration of action of the experimental agent, being less marked in the group treated with ultraviolet light and of high grade in the infected group. This fact alone shows that the agents used are not sufficiently selective to fulfil the requirements of the "ideal agent." The absence or moderate grade of hyperplasia in the animals treated with ultraviolet light, however, indicates that this agent, at least, is selective to a fairly high degree. Possibly megakaryocytes are sensitive to stimuli which in greater concentration affect the other elements also. The second agent, being much more intense in its effects, not only caused greater changes in circulating platelets and in megakaryocytes, but also stimulated the other elements of the bone marrow to marked hyperplasia.

### CONCLUSIONS

Increase in the number of circulating blood platelets produced by the use of ultraviolet light or by a specific bacterial infection is accompanied by increase in the number of megakaryocytes and by increased evidence of the formation of platelets on the part of the megakaryocytes, namely, Wright's figures, fragmentation of the cytoplasm and the appearance of "naked nuclei."

The mode of action of the agents used is by direct stimulation of the bone marrow, as evidenced by the tendency of the megakaryocytes to accelerated maturation and by the general hyperplasia of bone marrow. The agents seem to be at first stimulating and subsequently toxic to the bone marrow. The megakaryocytes respond earlier and are affected more profoundly than the other elements of the marrow.

The inclusion of other cells of the bone marrow within the cytoplasm of megakaryocytes has been observed. Whenever this feature is at all conspicuous, inclusion seems to be limited to the granulocytes, and the nuclei of the megakaryocytes show evidence of degeneration. For this reason, the phenomenon is regarded as an invasion of damaged megakaryocytes by active granulocytes rather than as phagocytosis of other blood cells by megakaryocytes.

# EXPERIMENTAL STUDIES ON THE RETICULO- ENDOTHELIAL SYSTEM

## V. THE INFLUENCE OF INDIA INK ON THE RATIO OF UREA IN THE BLOOD \*

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The liver in uremia shows, as a rule, considerable edema, with the fluid accumulating mainly between the trabeculae of the hepatic cells and the wall of the capillary. In the course of this process, changes of the Kupffer cells are met with, such as swelling, desquamation and alteration in the chromatin structure of the nucleus. These changes observed at autopsy suggested a possible relationship of the Kupffer cells to the disturbance in nitrogen metabolism, which is looked on as the basic factor in the development of the uremic symptoms. This possibility is the more probable as the liver is supposed to be the main seat of the formation of urea, and this substance, synthesized in the liver cells, reaches the circulating blood after having passed through the barrier of the Kupffer cells.

These considerations led us to investigate the effect of a blockade of the Kupffer cells on the urea content of the blood. India ink was used as the blocking material, and the experiments were carried out on mice, rabbits and dogs.

### EXPERIMENTAL OBSERVATIONS

The experiments on mice were made after determining the average urea content of the blood of the mouse after a fast of twenty-four hours. Determinations on ten mice showed variations of urea nitrogen from 9 to 15.4 mg. per hundred cubic centimeters of blood, the average being close to 14 mg. After injection of 0.5 cc. of india ink, diluted ten times, into a vein of the tail of each mouse, amounts were obtained that varied from 19 mg. to 33 mg., with an average of 26.5 mg. The determination of urea nitrogen was made four hours after the injection of india ink. It is obvious that such a substantial rise could not have been due to retention of the nitrogenous products of cleavage. The period of four hours is certainly too short to account for any considerable retention. Moreover, we carefully examined the kidneys of the ten

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\* From the Department of Pathology, United Israel Zion Hospital.

mice and ascertained that the glomeruli showed no embolic obstruction or other impairment.

In order to show that the rise in the blood urea after the injection of india ink was actually independent of renal function, we compared our observations with those obtained on mice poisoned with uranium nitrate. Seventy-two hours after the injection of uranium nitrate ten mice showed a rise in urea nitrogen, which varied in amounts from 20 to 50 mg., the average being 35.2 mg. Our next step was to inject india ink into animals that had previously been poisoned with uranium nitrate. The amounts obtained four hours later showed a wide range of the level of urea nitrogen in the blood, the lowest being 22 mg. and the highest 102 mg., with an average of 50 mg.

*Urea Nitrogen Content of the Blood Before and After Injection of India Ink and Uranium Nitrate*

Rabbit	After Fasting, Mg.	After Injection of		
		Ink, Mg.	Uranium Nitrate, Mg.	Ink, Mg.
1.....	17.0	20.0	92.0	..
2.....	15.5	21.0	52.0	..
3.....	14.5	21.5	44.0	..
4.....	13.5	24.0	70.0	..
5.....	16.0	21.6	49.0	..
6.....	13.0	25.0	34.0	..
Average.....	15.0	22.2	57.0	..
7.....	18.0	....	40.0	60
8.....	17.5	....	34.0	45
9.....	18.5	....	28.5	54
10.....	16.0	....	20.0	47
11.....	18.5	....	30.0	58
12.....	16.5	....	24.5	35
Average.....	17.5	....	31.0	50

All these experiments were made with small quantities of blood. In order to eliminate possible errors inherent in such procedures, we repeated the experiments on a series of rabbits. Our procedure was as follows:

The animals were made to fast for twenty-four hours. A sample of blood was taken to determine the normal rates of urea nitrogen, creatinine and non-protein nitrogen; 5 cc. of a 1 per cent solution of uranium nitrate was injected subcutaneously into six animals; the animals were fed lettuce; twenty-four hours later blood was taken, and 5 cc. of india ink twice diluted was injected intravenously; four hours later a third sample of blood was taken. The results obtained in this series were compared with those obtained in a second series of six rabbits into which india ink was first injected and then uranium nitrate. Three samples of blood were also taken in this series: one before the injection of india ink, one four hours later and one twenty-four hours after the injection of uranium nitrate.

The results of these experiments can be read in the table. Injection of india ink into the rabbits was followed by a rise in urea nitrogen

from a normal level of 15 mg. to 22.2 mg. Both figures represent the averages of six determinations. Changes in the creatinine and total non-protein nitrogen ratios were commensurate. When the injection of india ink was followed by the administration of uranium nitrate, a second considerable rise in the blood urea occurred, the figures varying from 34 to 92, with an average of 57.

In the reverse experiment in which the kidneys were damaged first, with a subsequent injection of india ink, the nitrogenous products in the blood reached somewhat lower figures. They varied between 35 and 60. Yet the injection of india ink in each case was followed within four hours by a new rise of the urea, considerably in excess of that produced by renal impairment alone.

We checked these experiments on dogs also, using four animals in these procedures. Incidentally, two of the animals had previously been splenectomized for some other purpose. We were somewhat surprised to see that the results obtained in the splenectomized dogs were different from those in the other two. Injection of india ink into the normal dogs produced a rise of the blood urea nitrogen from 20 to 24 mg. and from 21.5 to 26.5 mg., respectively. A second determination twenty-four hours later showed that the blood urea had returned to its original level. In the two splenectomized dogs the urea nitrogen was 20 mg. before the injection of india ink; four hours later, it was only 16 and 18 mg., respectively, while after twenty-four hours it dropped in both animals to as low as 12 mg.

#### COMMENT

Our experiments showed that injection of india ink into various laboratory animals elicits a temporary rise in the blood urea nitrogen, which disappears after twenty-four hours, at least in the dog. The rise is not due to impaired renal excretion, as was shown by the unaltered structure of the kidneys. This is also borne out by the observation that india ink injected into animals previously poisoned with uranium nitrate elicited a further rise. On the other hand, the highest ratio of blood urea in animals treated by injection of india ink could be still further augmented if the kidneys were impaired by poisoning with uranium nitrate.

Experiments by Rothmann and Sylla<sup>1</sup> also showed a rise of the nonprotein nitrogen of the blood after injection of india ink. Their results, however, are not quite comparable, because these authors injected the ink into the renal artery after removing the other kidney. The kidney receiving the injection, when examined from two to three days later, showed granules of ink in the glomerular capillaries and

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1. Rothmann and Sylla: *Ztschr. f. exper. Med.* **69**:356, 1930.

lesions of both glomeruli and tubular parenchyma. Rothmann and Sylla concluded, therefore, that the rise in nonprotein nitrogen observed in the blood after from two to three days was due to impairment of renal function.

We do not deny that lesions of the kidneys and subsequent retention of nitrogen can be produced by injection of ink into the renal artery. But we wish to emphasize the fact that no renal lesions are obtained if ink is given intravenously, as in our experiments. It is regrettable, moreover, that Rothmann and Sylla did not mention whether they examined the liver and spleen of their experimental animals. In our experience with injection of colloidal suspensions, we found that apparently not all of the material injected intra-arterially is filtered off by the capillaries. A fairly large amount passes through the capillaries and becomes electively stored in the reticulo-endothelial cells. Thus the results of Rothmann and Sylla seem to be derived from two widely different sources: from renal impairment and from interference with the reticulo-endothelial system.

If renal retention does not account for the rise in blood urea in our experiments, it seems logical to assume an excessive liberation of urea into the blood. This assumption at first seems inconsistent with the general belief that urea is manufactured by the hepatic cells, and there is no morphologic evidence to show that india ink is taken up by these cells; nor can we demonstrate any other direct interference of the ink with the parenchyma of the liver. The granules of ink are abundantly stored in the Kupffer cells, and it would seem that such storage may have certain effects on the function of these cells. On this basis we could attempt to explain the increase in blood urea by the assumption that the urea produced by the hepatic cells permeates more easily into the circulation if the Kupffer cells are incapacitated. This explanation, however, is far from satisfactory and is strangely contradicted by our observation on splenectomized dogs, in which india ink did not give rise to an increase of blood urea. This observation would rather suggest an increased function of the Kupffer cells and other biologically related elements, such as the splenic pulp. In the absence of the spleen a diminished response could be expected.

A report of experiments recently published by Buengeler<sup>2</sup> seem to shed considerable light on the nature of the metabolic changes discussed in this paper. Using the method of Warburg, Buengeler studied the gas exchange, glycolysis and production of lactic acid on surviving tissues. The tissues examined, however, were obtained from animals that previously had been given intravenous injections of india ink or of other

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2. Buengeler: Frankfurt. Ztschr. f. Path. **39**:426, 1930.

colloidal suspensions. He observed a remarkable increase in the consumption of oxygen which, as parallel histologic examinations showed, was simultaneous with progressive phagocytosis of the granules of ink by the Kupffer cells of the liver or by the reticulum cells of the spleen. Buengeler interpreted his results as indicative of an activation of the reticulo-endothelial cells due to stimulation by the foreign material introduced into the circulation. Such activation of the cells also suggests other metabolic processes, besides the increased consumption of oxygen, which are most likely to yield nitrogenous products through cleavage. It seems logical to connect the increase of urea in the blood after the injection of ink with Buengeler's endothelial activation, particularly with enhanced function of the Kupffer cells and possibly of other reticulo-endothelial elements. In view of these observations it would seem to be indicated that in the future distinction must be made between an increase of blood urea due to urinary retention and that which is caused by increased activity of the mesenchymal cells.

Recent investigation by Fischer and Frommel<sup>3</sup> on experimental tuberculosis points in the same direction. These authors observed a rise in the consumption of oxygen of as much as 50 per cent during the period of incubation, while no rise in temperature was noted. These experiments confirmed previous clinical observations in afebrile tuberculosis (Grafe<sup>4</sup>), subacute bacterial endocarditis (Gessler<sup>5</sup>), the prodromal stage of measles or of varicella (Birk<sup>6</sup>) and the incubation stage of therapeutic malaria (Strieck and Wilson,<sup>7</sup> Bahn and Langhans<sup>8</sup>). In all these conditions the increased consumption of oxygen was independent of any rise in temperature. Grafe suggested an irritation of cerebral centers as a possible cause of the metabolic processes expressed by the rise in consumption of oxygen. In our opinion, however, it is more logical to explain the sudden increase of metabolic activities by an increased function of the reticulo-endothelial system, which is known to play the leading rôle in the processes of defense against infections. It was shown in previous papers<sup>9</sup> that the early stage of infection is characterized by changes of the reticulo-endothelial cells, which were interpreted as evidence of activation and increased function of the cells. The observations on the changes in metabolism

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3. Fischer and Frommel: *Ztschr. f. exper. Med.* **74**:646, 1930.

4. Grafe: *München. med. Wchnschr.* **67**:1081, 1920.

5. Gessler: *Deutsches Arch. f. klin. Med.* **144**:188, 1924.

6. Birk, quoted by Strieck and Wilson (footnote 7).

7. Strieck and Wilson: *Deutsches Arch. f. klin. Med.* **157**:202, 1927.

8. Bahn and Langhans: *Deutsches Arch. f. klin. Med.* **161**:181, 1928.

9. Goldzieher: *The Structure of Infectious Splenic Swelling*, *Arch. Path.* **3**:42, 1927. Goldzieher and Peck: *Experimental Studies on the Reticulo-Endothelial System: I. Response to Infection*, *Arch. Path.* **3**:629, 1927.

during the period of incubation impress us as additional evidence that any stimulation of the reticulo-endothelial system elicits reactive changes that are amenable to morphologic, serologic or biochemical demonstration. In our experiments with the injection of india ink this reaction became apparent by the increase of the nitrogenous products of cleavage in the blood.

#### SUMMARY

The urea nitrogen of the blood was studied in mice, rabbits and dogs following injection of india ink. Such injection elicited a rise of urea nitrogen, which could not be attributed to renal retention. An additional rise was produced by india ink when this was injected into animals the kidneys of which had previously been damaged by the administration of uranium nitrate. No rise was obtained if the india ink was injected into splenectomized dogs.

The rise in the blood urea after the injection of india ink is interpreted as the result of increased activity on the part of the reticulo-endothelial system.



# EXPERIMENTAL PATHOLOGY OF THE LIVER

## I. RESTORATION OF THE LIVER OF THE WHITE RAT FOLLOWING PARTIAL SURGICAL REMOVAL \*

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AND

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Since on the surgical removal of from 65 to 75 per cent of the hepatic parenchyma compensatory hyperplasia of the remaining lobes follows so rapidly, the questions arise (1) whether a liver so quickly restored performs the usual hepatic functions in a manner to make it comparable to a normal liver, (2) whether the glycogenic function and the bile-secreting function are as effective in the new parenchyma and (3) whether the defense mechanism, or its detoxifying activity, is any less effective in a recently restored liver than in a normal one. These questions, as well as many others, must be answered before knowledge of the liver is complete. Before physiologic or pathologic studies of this sort are possible, complete data must be available concerning such pertinent facts as: (1) when restoration begins following partial removal, (2) when it is most active, and (3) when it is complete, or when the normal ratio of the weight of the liver to the weight of the body is restored.

Although science has known for many years that rapid recovery follows either extensive chemical injury or partial surgical removal, so far as we know, no statistical approach has ever been made toward the solution of the factors in restoration. Such statistical data as concern (1) the ratio of the weight of the liver to the weight of the body during the period of restoration, (2) the rate of restoration and (3) the ratio of the weight of dry liver to the weight of moist liver at frequent intervals during the period of restoration have never been compiled.

In biologic studies of this sort, in which the variable factor, such as the weight of the liver in relation to the weight of the body, varies considerably in each animal, irregularities in the analysis of the data are unavoidable. Since the weight of the hepatic parenchyma in animals of comparable size may vary as much as 2 or 3 Gm., precision in the

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\* From the Division of Experimental Surgery and Pathology, the Mayo Foundation.

evaluation of such data as we have attempted to assemble can only be approached through the application of the statistical method. The exact technic of the chemist is not available for a study of this sort, and thus in order to avoid misinterpretation and unwarranted conclusions we have used a large number of animals and applied the statistical method.

A brief comment on terminology may not be out of place. Ever since the early recognition by Cruveilhier and Andral that the liver rapidly recovers from injury, the term "regeneration" has been employed to describe the process. The large bulk of experimental work which forms the basis for the present knowledge of the factors and the rates of regeneration of somatic tissue has been performed on invertebrate and lower vertebrate forms of life. In extensive experiments Roux, Driesch, Morgan, Child and Zeleny and many others have considered in detail the reaction of the organism to such injuries as the removal of a limb, a cranial appendage or a caudal appendage, or the removal of parts of a developing embryo in either the morula or the blastula stage. Under these conditions a reaction at the level of injury ensues and a new limb or a new tail develops. Various terms, such as "replacement of lost parts," "restitution" or "reparation," have been employed to designate the reaction of the organism, but the term "regeneration," according to Morgan, more accurately describes these processes. In such cases of regeneration active proliferation of cells invariably precedes differentiation. New tissues develop at the level of the injury, and after a period of active proliferation a new limb with its component parts is differentiated.

In the case of the mammalian liver one is dealing with a response in growth considerably different from that which ensues on the removal of the tadpole's tail. Following partial removal of the liver, a proliferation of cells does not ensue at the level of the cut; new lobes do not develop to take the place of those removed. There is, on the other hand, hyperplasia of the remaining lobes. There are no centers of cell proliferation, but generalized mitotic activity, coupled with cellular migration and differentiation, results in the formation of new lobules, and the preoperative weight of the liver is thus rapidly restored. Regeneration, as employed biologically, would involve, it seems to us, the replacement of a removed part, with rearrangement or reorganization of the old part. The term could well be applied to the recovery of a hepatic lobule from chemical injury as, for example, chloroform or carbon tetrachloride poisoning, in which there is not only reorganization of portions of the old lobule, but new cellular proliferation as well.

Accordingly, we have used the term "restoration" in this study of the mammalian liver in order that we might distinguish the reaction

following partial surgical removal from that which ensues on chemical injury.

An extensive bibliography on regeneration of the liver was published by Fishback<sup>1</sup> in 1929. We have not, therefore, attempted to review the literature in this report.

#### METHOD OF STUDY

Rats were selected for this study not only because of their availability in sufficient numbers to warrant extensive use, but because they withstand surgical procedures, and because postoperative mortality among them is usually low. Their natural resistance to infections makes them desirable surgical risks.

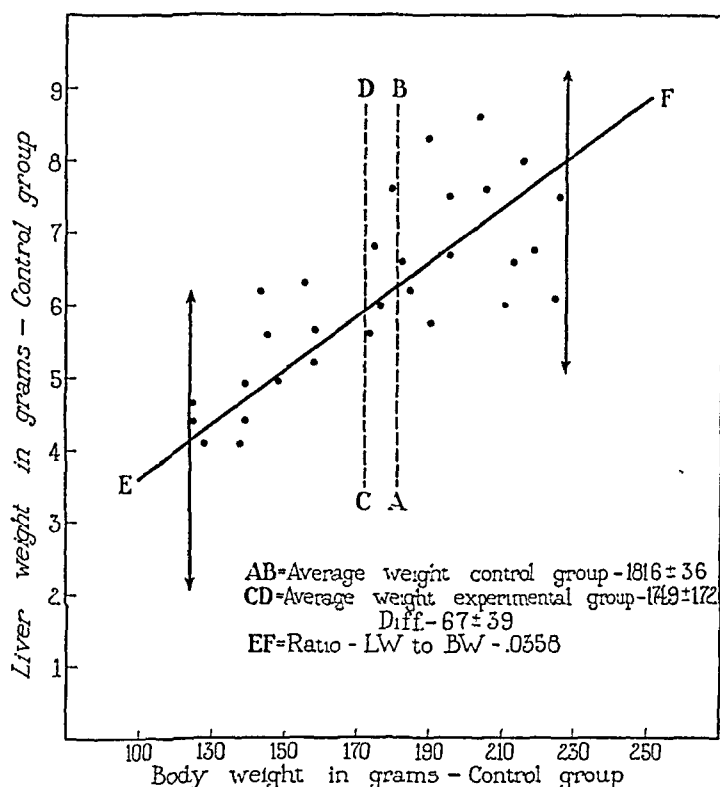


Fig. 1.—Distribution of the control group of animals in regard to weights of liver plotted against weights of body.

Prior to the surgical removal of a portion of the rat's liver preliminary to a study of restoration we wished to determine the mean weight of the liver and the ratio of its weight to the weight of the body, within our experimental group. Accordingly, fifty rats, ranging in weight from 90 to 275 Gm., were killed by exsanguination, and the weights of their livers were plotted against the weights of their bodies. Within the limits of weights of body of approximately 125 and 225 Gm. weights of the liver grouped themselves into a relatively linear distribution (fig. 1). If animals weighed less than 125 Gm., the weights of the liver were

1. Fishback, F. C.: Arch. Path. 7:955, 1929.

relatively higher; whereas if they weighed more than 225 Gm., there was a tendency for the weights of the liver to be relatively lower. Accordingly, throughout our entire study we restricted our observations to animals ranging in weight from 125 to 225 Gm. and determined the figure 0.0358 as being the percentage of the weight of the liver in relation to the weight of the body. This is considerably lower than the percentages listed in the tables from the Wistar Institute,<sup>2</sup> and somewhat lower than those listed by Jackson<sup>3</sup> in 1913 and by Rous and McMaster<sup>4</sup> in 1924. We are unable to explain this discrepancy, but believe it to be correlated with diet or perhaps with the strain of the rats of our colony.

#### TECHNIC OF PARTIAL REMOVAL

The liver of the rat is a firm, dark red organ and according to Hunt<sup>5</sup> is composed of four main lobes. The median lobe is cleft by a longitudinal fissure, which divides it into a right central and left central lobe. The right central lobe is flanked by the right lateral lobe, which is cleft transversely by a fissure dividing it into two smaller lobes, the posterior one of which caps the anterior pole of the right kidney. The left lateral lobe is a large lobe and lies immediately behind the left central lobe. The caudate lobe is likewise cleft by a transverse fissure and comprises thereby two smaller lobes which lie within the curvature of the stomach and the lesser omentum. Anatomically, the median lobe, with its two central portions, together with the left lateral lobe, forms somewhat of a unit which lends itself well to surgical removal.

All operations were carried out with the animals under ether anesthesia, and careful asepsis was maintained throughout. Through a median-line incision reaching 3 or 4 cm. posteriorly from the xiphoid process of the sternum the large median lobe of the liver, with the left lateral lobe, was easily delivered, securely ligated by coarse linen and then excised. In this way portions of the hepatic parenchyma ranging in extent from 65 to 75 per cent of the total liver were removed, leaving within the peritoneum the right lateral lobe and the small caudate, or spigelian, lobe. The abdomen was closed with two layers, one suture of linen being used; the peritoneum and the abdominal muscles were closed in the first layer and the integument in the second. There was no special postoperative care, except that in place of water the animals had access to a 20 per cent solution of dextrose for the first day, after which the normal diet and water were provided. The full diet consisted of known quantities of yellow corn meal, linseed oil meal, crude casein, alfalfa meal, powdered skim milk and salts.

Immediate postoperative complications were not common, and yet, owing essentially to lesions of the lung, which one often encounters in laboratory rats, about 25 per cent of the 220 hepatectomized animals that form the basis for this report died. Figure 2 represents the distribution in regard to weight of body and actual amount of hepatic parenchyma removed of the 104 animals that survived and constituted the experimental series for data on the weight of the moist liver during restoration. The mean weight of the experimental series was 174.9

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2. Donaldson, H. H.: *The Rat: Data and Reference Tables for the Albino Rat (Mus Norvegicus Albinus)*, Philadelphia, Wistar Institute, 1924.

3. Jackson, C. M.: *Am. J. Anat.* **15**:1, 1913.

4. Rous, Peyton; and McMaster, P. D.: *J. Exper. Med.* **39**:425, 1924.

5. Hunt, H. R.: *A Laboratory Manual of the Anatomy of the Rat*, New York, The Macmillan Company, 1924.

$\pm 1.72$  Gm., and since the mean weight of the control group from which we derived our mean ratio of weight of liver to weight of body was  $181.6 \pm 3.6$  Gm., a difference of only  $6.7 \pm 3.9$  Gm., we felt reasonably sure that we were dealing with a comparable group of animals and that our data would be reasonably accurate. The mean amount of liver removed from the entire group that survived and formed the basis for the computation was  $4.43 \pm 0.047$  Gm. Since the mean preoperative weight of the liver of the experimental group was computed at 6.27 Gm., we had removed on the average 70.6 per cent of the entire liver. A few animals were killed at frequent intervals during the first eighteen hours after operation for a study of cytologic changes in the residual lobes; others were killed

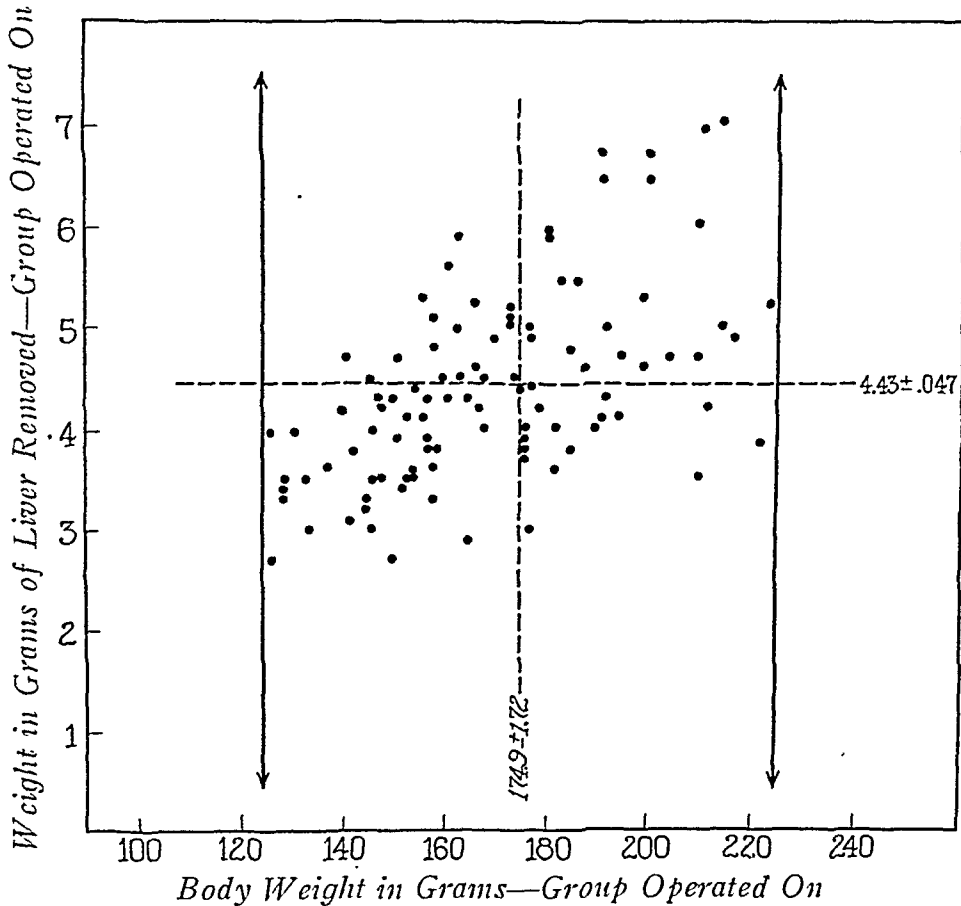


Fig. 2.—Distribution of the experimental group of animals in regard to the weights of liver removed at partial hepatectomy, plotted against weights of body.

at longer intervals during the entire period of restoration for determinations of the weight of the liver and the weight of the body, as well as for cytologic data. Experimental animals were killed by exsanguination at each of the following intervals after partial removal of the liver, up to four weeks, when restoration was considered as essentially complete: from sixteen to twenty-four hours, from twenty-four to forty-eight hours, from forty-eight to seventy-two hours, from ninety-six hours to seven days, from seven to fourteen days, from fourteen to twenty-one days and from twenty-one to twenty-eight days.

Although the weight of the liver in our control group was found to be 0.0358 of the weight of the body, it was nevertheless desirable to obtain a linear equation

which would best describe one variable in terms of the other; that is, weight of the liver in terms of weight of the body. Accordingly, for all animals of the experimental series, an equation expressing weight of the liver in terms of the variable weight of the body was computed from a knowledge of means, standard deviations and correlation coefficients. The linear equation computed on the basis of the data assembled from thirty animals of the control series was determined as shown in footnote <sup>6</sup> and as shown graphically in figure 1.

Comparable data were assembled for the entire series of 104 animals. Pre-operative weight of body and weight of liver, the amount of hepatic parenchyma removed at operation, the postoperative weight of body and liver and the net increase in amount of hepatic parenchyma for a given period were all determined, with their probable errors. The data on moist weight of liver assembled during the period of restoration for the 104 animals have been condensed in table 1.

6.  $x$  equals weight of body;  $y$  equals weight of liver; number of animals, 30.

$$v1x \text{ (mean)} = \frac{5448.4}{30} = 181.6133$$

$$v2x = \frac{1,014,902.63}{30} = 33,830.087$$

$$u2x = v2x - (v1x)^2 = 851.52$$

$$\delta_x \text{ (standard deviation of } x) = \sqrt{u2x} \text{ or } 29.18$$

$$v1y \text{ (mean)} = \frac{195.16}{30} = 6.505$$

$$v2y = \frac{1306.2744}{30} = 43.54248$$

$$u2y = v2y - (v1y)^2 = 1.2274$$

$$\delta_y \text{ (standard deviation of } y) = \sqrt{u2y} \text{ or } 1.1078$$

$$vxy = \frac{36032.906}{30} = 1201.096$$

$$u_{xy} = v_{xy} - (v1x)(v1y) = 1201.096 - 1180.460 = 20.636$$

$$r_{xy} \text{ (correlation coefficient)} = \frac{u_{xy}}{\sigma_x \sigma_y} = \frac{20.636}{32.325} = 0.6383$$

$$\text{Formula: } y - \text{mean } y = r_{xy} \frac{(\sigma_y)}{\sigma_x} (x - \text{mean } x)$$

$$y - 6.505 = 0.6383 \frac{1.1078}{29.18} (x - 181.61) = 0.02423 (x - 181.61)$$

$$= 0.02423x - 4.400$$

$$y = 0.02423x + 2.105$$

$$\text{or } y = 0.024x + 2.1 - \text{working formula}$$

The standard deviation of the weight of the liver about this regression line was computed by the formula

$$\delta_{y,x} = \sqrt{\delta_y^2(1-r_{xy}^2)} \text{ in which } r \text{ is the correlation coefficient}$$

$$= \sqrt{(1.1078)^2 (1-0.6383^2)}$$

$$= 0.8528 \text{ or the standard deviation of the weight of the liver.}$$

The probable error of the mean weight of the liver is equivalent to

$$0.6745 \frac{0.8528}{\sqrt{n-1}}$$

Accordingly, the preoperative mean weight of the liver for each group of animals was computed on the basis of the formula  $y = 0.024x + 2.1 \pm \frac{0.5752}{\sqrt{n-1}}$  in which

$x$  equals weight of body and  $y$  equals weight of liver. For illustration, in the first group of fourteen animals in the experimental series, killed from sixteen to twenty-four hours after surgical removal of the liver, the mean weight of the liver was computed as follows: Mean weight of body was  $174.5 \pm 5.36$  Gm. Mean weight of liver was  $0.024 \times 174.5 + 2.1 \pm \frac{0.5752}{\sqrt{13}} = 6.288 \pm 0.1595$ . The actual weights of the hepatic parenchyma removed surgically were determined; the mean weight of the liver removed, with its probable error, was  $4.19 \pm 0.1242$  Gm.

Besides the series of animals the data on which are compiled in table 1, a second series of animals consisting of sixty-nine rats was used to determine the weight of the dry liver during restoration. The animals were killed by exsanguination and after taking the weights of the moist livers the livers were weighed after three weeks of thorough desiccation in an incubator at from 98 to 100 C. Computation of weights of both the moist and the dry livers of a control group, within the limits of body weight of 125 and 225 Gm., showed that in our control group with a mean weight of the body of  $177.1 \pm 7.870$  Gm., the weight of dry liver was 28.7 per cent of the weight of moist liver and 1.042 per cent of the weight of the body.

In this series, as in the other, the preoperative weight of liver was determined by the formula  $y = 0.024 \times + 2.1 \frac{0.5752}{\sqrt{n-1}}$  in which  $x$  is the weight of the body,  $y$  the weight of the liver and  $n$  the number of animals used. The preoperative weight of dry liver was then computed on the basis of the control observation that the weight of dry liver is 28.7 per cent of the weight of moist liver. The percentage of the preoperative weight of dry liver in relation to the weight of the body was thus determined for all experimental animals in this series, as well as that of the weight of the residual dry hepatic parenchyma remaining in the peritoneum after operation. The postoperative weight of the body and weight of the dry liver with their probable errors were computed during the period of restoration at the intervals noted and the increase of weight of dry liver, in grams, was determined. The data assembled from the experimental series on the weight of dry liver during restoration have been condensed in table 2.

#### EXPERIMENTAL OBSERVATIONS

The rats ordinarily withstood operation exceedingly well, and surgical mortality was low. Postoperative mortality, however, was higher, owing essentially to the pulmonary complications so often encountered in these animals; mortality rates as high as 25 per cent occurred. There were no indications that any of the normal physiologic processes were disturbed, except for temporary loss of weight. Occasional gross indications of anemia were manifest, but blood counts taken on a number of rats during the period of restoration did not reveal variation other than would be encountered in the normal rhythm. Loss of weight of varying duration always accompanied partial hepatectomy. That this loss was not due to hepatectomy alone was concluded from an experiment, the graph of which is shown in figure 3. Three series of

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The difference between the mean weight of the liver prior to operation and the mean weight of the liver removed at operation constituted the mean weight of the remnant of hepatic parenchyma remaining within the body; this was found to be  $2.098 \pm 0.2021$  Gm., in which the probable error of the difference was determined by the square root of the sum of the probable errors of the original mean weight of the liver and of that of the actual parenchyma removed. When the animals were killed for data on restoration, the actual weights of the liver at death were taken, and the mean with its probable error was determined. The difference between the mean weight of the remnant of the liver at operation and the mean weight of the liver at death constituted the actual increase during restoration; this was  $0.752 \pm 0.2290$  Gm., in which the probable error of the difference was computed as indicated.

TABLE 1.—Mean Weights of Body and of Moist Liver Before Partial Hepatectomy and at Intervals During Restoration

Group	Animals	Lapse of Time After Operation Before Animals Were Killed	Mean Weights									
			Before Operation			At Time of Partial Hepatectomy			At Time of Death		During Restoration	
			Body, Gm.	Liver, Gm.	Percentage of Weight of Liver*	Liver Removed, Gm.	Liver Remaining, Gm.	Liver, Gm.	Body, Gm.	Liver, Gm.	Hepatic Increment (Moist) Gm.	Percentage of Weight of Liver*
Control	30	.....	181.6 ± 3.65	6.50 ± 0.1389	3.58	4.10 ± 0.1242	2.008 ± 0.2021	164.1 ± 5.32	167.4 ± 3.89	2.85 ± 0.1077	0.752 ± 0.2200	1.736
1	14	16-24 hr.	174.5 ± 5.36	6.28 ± 0.1595	3.59	4.45 ± 0.1510	2.267 ± 0.2196	179.9 ± 4.06	177.9 ± 4.26	3.58 ± 0.1336	1.313 ± 0.2570	1.989
2	14	24-48 hr.	192.4 ± 1.69	6.71 ± 0.1595	3.49	4.62 ± 0.1653	1.710 ± 0.2434	159.9 ± 5.69	157.3 ± 7.200	4.47 ± 0.1181	2.760 ± 0.3706	2.765
3	15	48-72 hr.	176.3 ± 5.25	6.33 ± 0.1537	3.59	4.25 ± 0.1499	1.830 ± 0.2110	146.8 ± 4.38	150.5 ± 4.390	4.51 ± 0.1227	2.690 ± 0.3440	3.072
4	16	4-7 days	165.4 ± 3.91	6.07 ± 0.1485	3.67	4.75 ± 0.1706	1.570 ± 0.2335	166.9 ± 5.44	167.7 ± 3.800	5.88 ± 0.2060	4.319 ± 0.3539	3.523
5	14	7-14 days	184.5 ± 4.47	6.32 ± 0.1595	3.43	4.24 ± 0.1405	1.850 ± 0.2044	171.9 ± 3.75	167.7 ± 3.800	6.23 ± 0.2013	4.380 ± 0.2868	3.624
6	16	14-21 days	166.5 ± 4.29	6.09 ± 0.1485	3.66	4.57 ± 0.1208	1.534 ± 0.1612	174.4 ± 5.89	167.7 ± 3.800	6.79 ± 0.2451	5.240 ± 0.3158	3.893
7	15	21-28 days	167.7 ± 4.57	6.12 ± 0.1537	3.65							

\* In relation to weight of body.

TABLE 2.—Mean Weights of Body and of Dry Liver Before Partial Hepatectomy and at Intervals During Restoration

Group	Animals	Lapse of Time After Operation Before Animals Were Killed	Mean Weights									
			Before Operation			At Time of Partial Hepatectomy			At Time of Death		During Restoration	
			Body, Gm.	Liver (Moist), Gm.	Percentage of Weight of Liver (Dry)*	Liver (Dry), Gm.*	Liver Removed, Gm.	Liver (Dry) Remaining, Gm.	Body, Gm.	Liver (Dry), Gm.	Hepatic Increment (Dry), Gm.	Percentage of Weight of Liver†
Control	8	.....	177.1 ± 7.870	6.430 ± 0.2090	1.845	1.845	4.90 ± 0.1599	0.4614	167.4 ± 9.002	0.9707 ± 0.0736	0.5093	0.5780
1	4	10-24 hr.	183.7 ± 8.800	6.508 ± 0.3321	1.867	1.867	4.95 ± 0.1169	0.5392	177.9 ± 4.226	1.0561 ± 0.095	0.5169	0.5060
2	4	24-48 hr.	198.3 ± 8.190	6.859 ± 0.3321	1.968	1.968	4.80 ± 0.2240	0.4290	157.3 ± 7.200	1.2387 ± 0.063	0.8297	0.8000
3	9	48-72 hr.	174.8 ± 6.600	6.295 ± 0.2033	1.806	1.806	4.40 ± 0.2260	0.5031	150.5 ± 4.390	1.2380 ± 0.033	0.7299	0.8190
4	13	4-7 days	168.9 ± 3.970	6.153 ± 0.1660	1.765	1.765	5.20 ± 0.2220	0.3949	173.3 ± 4.650	1.8677 ± 0.097	1.4728	1.048
5	7	7-14 days	186.5 ± 4.520	6.576 ± 0.2348	1.887	1.887	4.30 ± 0.1282	0.4890	167.7 ± 3.800	1.6712 ± 0.045	1.1822	0.995
6	14	14-21 days	162.7 ± 4.150	6.004 ± 0.1595	1.723	1.723	4.82 ± 0.1524	0.3673	156.6 ± 5.420	1.7094 ± 0.071	1.3421	1.091
7	10	21-28 days	154.2 ± 4.110	5.800 ± 0.1917	1.664	1.664						

\* 28.7 per cent of moist.

† In relation to weight of body.



rats of comparable ages and weights, including a normal group not subjected to operation, a group in which simple laparotomy was performed, and a group consisting of sixteen partially hepatectomized animals, were maintained on identical diets and weighed daily for thirty days. Daily fluctuations were encountered in the group not operated on, but a moderate increase in weight took place during the period of observation. In both the series subjected to simple laparotomy and the series partially hepatectomized a loss of weight involving from 12 to 15 per cent of the original weight was encountered during the first ten days following operation. Return to normal weight was slightly more rapid if laparotomy was performed than if hepatectomy was done, and at the end of thirty days, the period selected as ordi-

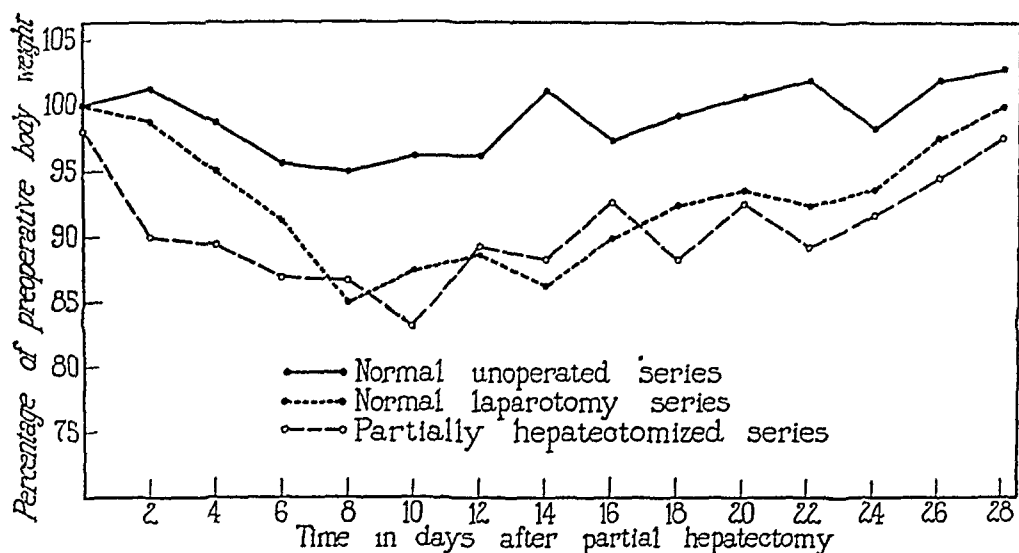


Fig. 3.—Body weights for twenty-eight days in (a) normal series, not operated on, (b) normal series subjected to laparotomy and (c) partially hepatectomized series.

narily required for complete hepatic restoration, both series had practically returned to their preoperative level. Consequently the loss of weight sustained in our entire experimental series was probably correlated more with operative procedure than with hepatic insufficiency. Anesthesia in itself bore no relation to loss of weight of the body.

Figure 4 shows isolated stages in the restoration of the liver at intervals, and figure 5 indicates graphically the percentage of removal in our entire experimental group together with the percentages of restored hepatic parenchyma which we encountered, at the intervals noted, during the entire period.

It is clear from a study of the data, in tables 1 and 2, on the weights of moist and dry liver during the period of restoration, and from the graph of the increase in weight of the liver in each 100 Gm.

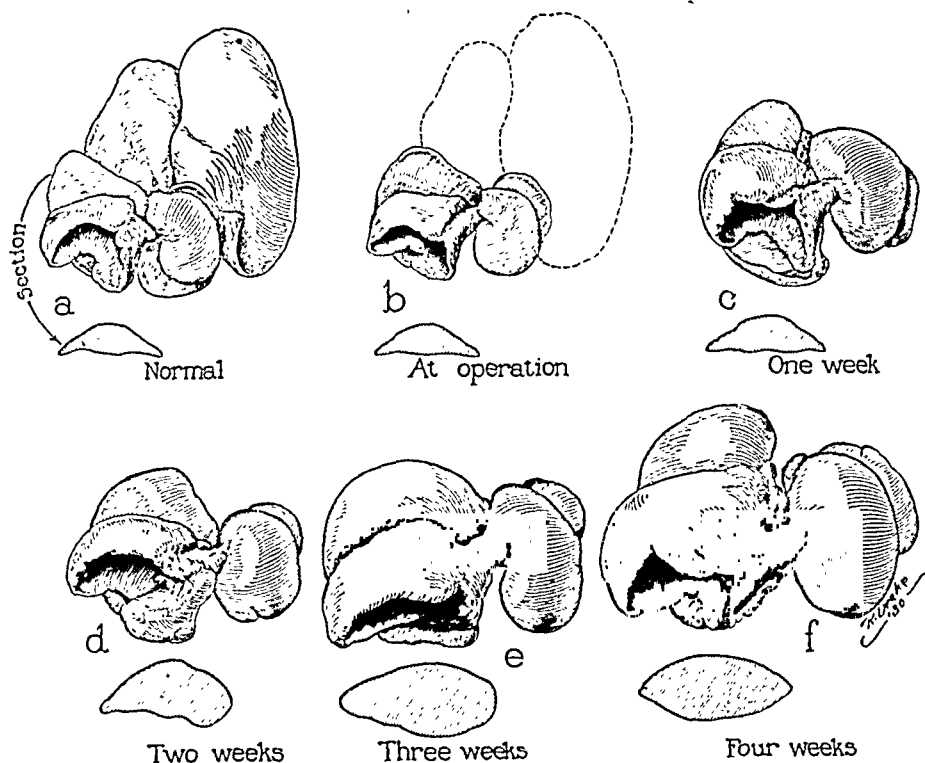
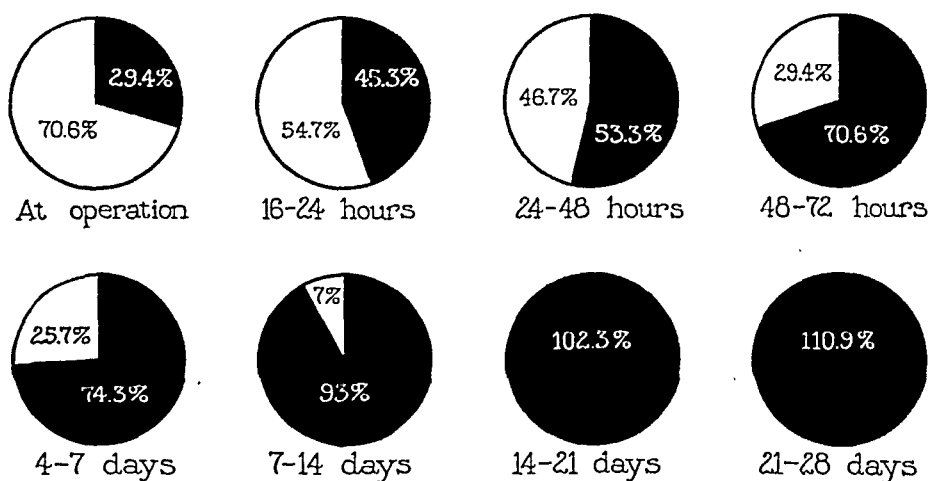


Fig. 4.—Liver of the normal rat at operation and at weekly intervals during restoration.



Restoration of the liver following partial hepatectomy

Fig. 5.—Percentage of liver removed at operation in relation to total weight of liver and percentages of restored weights of liver at intervals during restoration.

of body weight during the period, that recovery from partial removal does not take place by uninterrupted continuous growth (fig. 6). The growth rather is cyclic, and thus comparable to many other biologic phenomena.

The initial response by the hepatic remnant was slight hypertrophy of both cytoplasmic bodies and nuclei. Mitosis began during the latter part of the first day, but division of cells was not active until the second and third days, when the most active increase in the liver, for

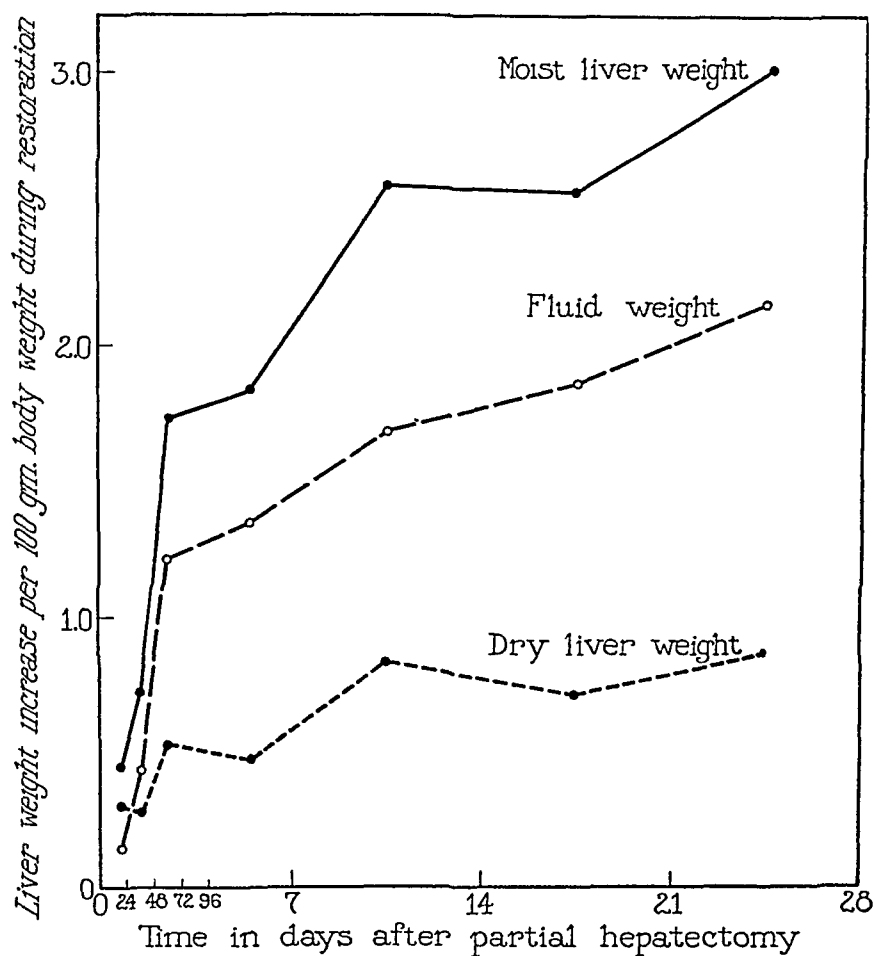


Fig. 6—Increase (grams) in weight of moist liver, weight of fluid and weight of dry liver during restoration.

each 100 Gm. of body weight, took place. With the increase in actual dry parenchyma the fluid intake was most marked during the second and third days, so that the percentage of the daily increment for this period reached 136. During the fourth, fifth and sixth days the increase in parenchyma fell, and the fluid intake was appreciably less, so that the percentage of the increment reached as low as 2. Following this period of relative inactivity a second, but less marked, period of growth occurred, during the second week, when the percentage of the

daily increment was computed at 8. A period of lesser activity followed the second wave, so that the daily percentage of the increment during the latter part of the period of restoration fell as low as 1. Figure 7 shows the percentage of restored weight of liver in relation to weight of body at intervals during the entire period, and it is clear that as far as the ratio of weight of the liver to weight of the body is concerned, restoration is essentially complete by the tenth day. The ratio of weight of dry liver to weight of body (0.0103) and the ratio of weight of moist liver to the weight of body (0.0358) were obtained at that time. But since weight of the body was considerably below the preoperative level during the period from the tenth to the fourteenth days, restoration could not be said to be complete as soon as the normal preoperative ratios had been regained.

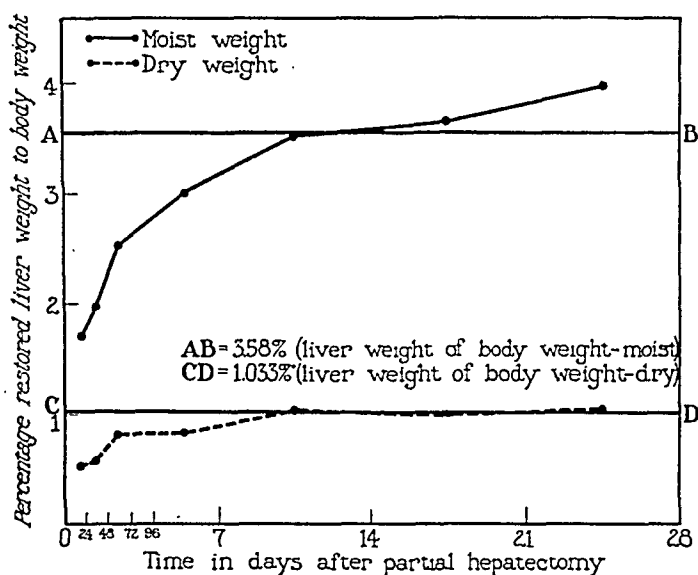


Fig. 7.—Percentages of weight of dry liver and weight of moist liver in relation to weight of body during restoration.

Following partial removal there is a rapid rise in the percentage curve of dry weights in relation to moist weights, reaching the peak of 34.1 at the end of the first day. This is considerably higher than the normal ratio of 28.7, and is probably due to two factors: a certain increase in actual parenchyma and loss of water pursuant to operation. Increase of fluid was noted at the end of the first day; it was greater than the increase of parenchyma and the percentage dropped to 27.3 by the sixth day. The rapid increase in hepatic parenchyma which occurred between the sixth and the tenth days again raised the percentage to 31.7; and then, owing again to increase of fluid, the percentage of dry weight in relation to moist weight was reduced to a figure below that accepted as the normal.

Thus the rate of restoration, although somewhat retarded during the first day, reached its peak during the third day, when the average daily increase of the liver for each 100 Gm. of body weight was approximately 1 Gm. This rapid rate was followed by a decline in acceleration, when daily increases as low as 0.4 Gm. for each 100 Gm. of body weight were recorded. A brief positive acceleration in the rate soon followed, reaching only 0.15 Gm. a day; the rate then gradually dropped, so that during the concluding days of the period of

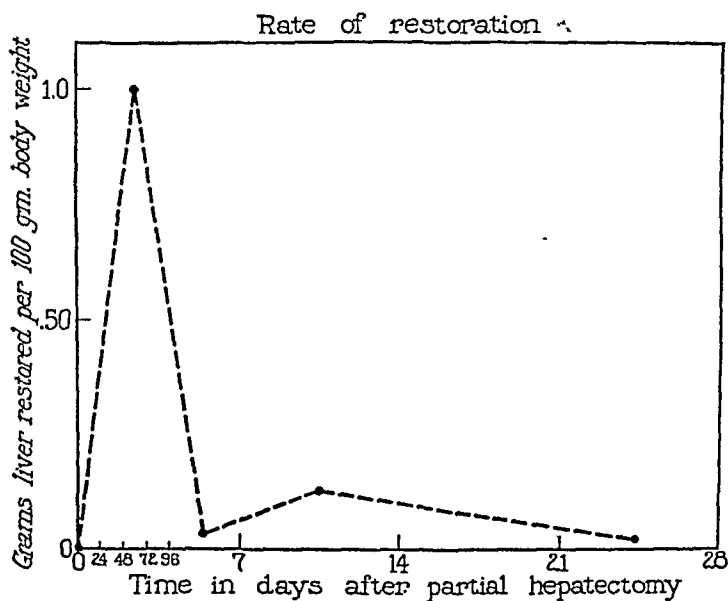


Fig. 8.—Graph of the rate of restoration of the liver, for each 100 Gm. of weight of body during restoration.

observation a rate of restoration of only 0.03 Gm. of hepatic parenchyma for each 100 Gm. of body weight was recorded (fig. 8).

#### COMMENT

The data in table 1 indicate that there was considerable uniformity in the weights of the bodies and in the weights of the livers of the control animals and of the animals killed at stated intervals during the period of restoration. Furthermore, in so large a series of cases of partial hepatectomy it is of interest to note the constant amount of hepatic parenchyma removed from animals of these seven groups, as indicated in the sixth column of table 1. In some cases, as in group 7, in which the mean weight of the body and the mean weight of the liver were lower than in certain others, an amount of parenchyma was removed equal to or greater than that taken from animals with larger livers. In those groups a smaller hepatic remnant formed the basis for the ensuing restoration.

The onset of restoration occurred during the latter part of the first twenty-four hours after operation. Mitotic figures were encountered, although they were not abundant—and yet an actual mean parenchymal increase of 0.752 Gm. occurred in the group of fourteen animals—during the first twenty-four hours. There was a certain amount of nuclear and cytoplasmic hypertrophy during this early period, which with the slight mitotic activity no doubt accounts for the increment. On the basis of body weight, the increment of moist liver was 0.458 Gm. for each 100 Gm., whereas 0.304 Gm. was the corresponding increase of dry parenchyma. Of the moist increment during this early period, tissue fluids constituted only 33 per cent, a figure considerably lower than the normal fluid content (71.3 per cent) and indicating strikingly a marked decrease in the water balance following partial hepatectomy.

During the second day, following partial removal, although a 60 per cent increase in moist parenchyma was recorded, this was found to be mainly fluid, for a slight decrease in dry parenchyma for each 100 Gm. of body weight was encountered. During the third day the percentage of the increment reached the highest level attained during the entire period of restoration, 136 per cent of the total restored liver at the end of the second day. The percentage of the dry increment was 61, and the fluid content was more nearly that of normal liver.

Following this rapid increase in the rate of restoration and in the amount of restored liver there was marked cessation in hepatic activity. The total restored weight of dry liver for each 100 Gm. of body weight during the period from the fourth to the seventh days was less than that recorded at the end of the third day, owing essentially to an increase in body weight. The percentage of the restored weight of liver in relation to weight of body during this period, however, was 0.03072, a figure considerably higher than that at the end of the third day, indicating that the ratio was fast approaching the figure accepted as the normal for our experimental group.

During the second week, a second impetus to restoration occurred, so that a percentage of weight of liver in relation to weight of body of 0.03523 was recorded. This was essentially the ratio that we accepted for our control series, and since the actual dry parenchyma was essentially comparable in weight with that of a normal rat of corresponding size one may say that restoration was complete at this time. Although there were frequent fluctuations in the weight of the liver during the latter part of the four weeks of observation, the changes were due to the intake of fluid rather than to hyperplasia. In fact, the greatest increase of dry parenchyma was attained during the second week after removal, and the ratio of dry weight of parenchyma to

body weight was 1.048, a figure somewhat higher than that accepted for our control group. The moist weights, it will be noticed, continued to show an increase after the second week. Although the normal ratio of moist weight of liver to weight of body was essentially restored by the fourteenth day, an increase in weight of moist liver actually ensued for the remaining two weeks of the period of observation. This increase was again due to the intake of fluids. During the second week the weights recorded for the moist and dry restored liver showed a content of fluid of 64 per cent. During the third week the content of water rose to 72 per cent, the figure accepted as essentially normal for our control group, and maintained that level throughout the period of observation.

The manifestations of cyclic restoration in the liver were of interest. At the end of the first day the dry hepatic parenchyma, on the basis of 100 Gm. of body weight, was 67 per cent of the moist weight, showing a fluid content of only 33 per cent. During the second day, although a marked increase in actual parenchyma had taken place, its dry weight represented only 40 per cent of the moist weight, indicating marked intake of fluid. At the end of the third day, during which the percentage of the actual increment reached a peak, the weight of dry parenchyma further dropped to 30 per cent of the moist weight, again indicating marked intake of fluid. During the interval between the fourth and the seventh days, when the daily percentage of the increment dropped exceedingly low, the hepatic cells continued to take up water, so that the percentage of dry weight to moist weight fell to 26.4. Between the seventh and the fourteenth days we encountered a further increase in cellular activity. The daily percentage of the increment had increased, mitosis was frequent, and the percentage of the fluid, which had reached its maximum during the period from the fourth to the seventh day, fell to 62. During the third and fourth weeks the cyclic changes were less marked. The normal water balance had been restored, there were no marked increases in the dry parenchyma, and the percentage of the dry weight in relation to moist weight hovered around 28.7, the figure accepted as the normal in our control group.

The greatest impetus to restoration occurred during the third day when the percentage of the increment reached 136.

These observations are considerably in accord with those of Zeleny,<sup>7</sup> who studied the rate of regeneration of the tail of *Rana clamitans*. He showed that the rate was slow at first, but soon increased rapidly to a maximum three or four days following surgical removal. The

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7. Zeleny, Charles: Illinois Biol. Monog. 3:1, 1916.

decline in the rate from the maximum was rapid at first, and then somewhat more gradual until zero was approached toward the end of the period of regeneration. To be sure, the gap between the caudal appendage of a cold-blooded amphibian and the liver of a warm-blooded mammal is phylogenetically great, yet this identity in rate of recovery following removal is biologically significant. Zeleny did not encounter positive acceleration after the initial acceleration of the fourth day, and the negative acceleration recorded by him was somewhat more gradual than the one recorded here for the liver. During the first four days the material present at the level of the injury to the amphibian tail was composed of cells that had migrated from residual tissue, and mitosis did not begin until after the fourth day. In the liver of the mammal, on the other hand, there was no migration of cells, as far as we know, except perhaps slight infiltration of the mesenchyme, and mitosis was well under way at the end of the first day and continued rapidly during the first few days. Zeleny stated that cellular differentiation impeded the rate of regeneration. A rapid rate, following soon after operation, gave rise to a zone of undifferentiated cells. As these transformed and assumed functional characteristics, as muscle or connective tissue fibers, the rate in further regeneration sharply declined, and negative acceleration was at its height. We have no data, physiologic or cytologic, that differentiation induced the marked negative acceleration encountered soon after the third day in the restoration of the liver of the rat. The cytologic appearance suggested activity of both parenchyma and biliary epithelium. Budding bile ducts, proliferating mesenchyme of the portal spaces and dividing cells of the parenchyma all contributed to the rapid increase in weight. Zeleny did not encounter a second impetus to restoration such as our figures have disclosed. Zeleny further showed that the rate of a second regenerative process somewhat exceeds that of the first regeneration following amputation of the tail. He has further shown that within certain limits the rate of regeneration from an injured surface is not retarded by simultaneous regeneration of parts in other regions of the body. It would be interesting to know whether additional injury to the mammal, such as splenectomy or nephrectomy, would modify the rate or the extent of restoration of the liver.

#### SUMMARY

Studies are reported on the rate and extent of restoration of the liver of the white rat following partial surgical removal. Moist weights and dry weights of the hepatic parenchyma have been taken on a series of rats killed at intervals during the period of restoration, and the assembled data have been condensed in tables 1 and 2.



Restoration began during the latter part of the first day following the removal of 70 per cent of the liver, and the preoperative ratio of weight of the liver to weight of the body was essentially restored at from the tenth to the fourteenth day. The more rapid rate of restoration took place during the third and fourth days. Since transient loss of body weight ensued on operation and was not essentially recovered until after from twenty-five to twenty-eight days, hepatic restoration was determined as complete at this time.

The liver, during the restorative period, disclosed cyclic activity. The most rapid restoration, as evidenced by mitosis and dry weights, occurred during the third day. In the period from the fourth to the seventh days the liver was less active cytologically, except for a marked increase in the intake of fluids. The period from the seventh to the fourteenth days was marked by a further increase in the amount of parenchyma and by the essential restoration of the normal ratio of the weight of moist and that of dry liver to the weight of the body. Further increase in the weight of the liver after the fourteenth day was essentially due to an increased intake of fluid.

# INDIVIDUALITY DIFFERENTIALS IN STRAINS OF INBRED RATS\*

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PHILADELPHIA

In a previous paper,<sup>1</sup> we reported on transplantation of tissues in relation to individuality differentials in two strains of rats that had been inbred by Dr. King for many years and through many consecutive generations by the process of mating brother and sister rats. The majority of the rats used in these earlier experiments had been inbred through from thirty-seven to thirty-eight generations, while a smaller number had been inbred through from forty-six to forty-seven generations. In these investigations we obtained the rather unexpected result that notwithstanding the long-continued close inbreeding the individuality differentials of the inbred rats had not become to any marked degree more similar to each other, although there was a distinct indication that slight progress toward greater similarity of the differentials had been accomplished. These results were in marked contrast to those found by Loeb and Wright<sup>2</sup> in transplantation of tissues in inbred strains of guinea-pigs. Here the individuality differentials of the members of an inbred strain had become much nearer to each other than the average individuality differentials of ordinary noninbred guinea-pigs or of inbred rats of strain A or strain B, although the inbreeding of the guinea-pigs had been continued through a much smaller number of generations. We suggested that the difference between the behavior of rats and guinea-pigs might be due to the selection in the case of the rats of the most vigorous individuals in each litter for further propagation of the strain. It was also conceivable that a greater number of spontaneous mutations take place in the rat than in the guinea-pig. In addition there is the possibility that the litters belonging to the same inbred strain between which the exchange of tissues or organs took place happened, in the case of the rats, to be further distant from each other than in the case of the guinea-pigs.

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1. Loeb, Leo, and King, Helen D.: *Am. J. Path.* **3**:143, 1927.

2. Loeb, Leo, and Wright, Sewell: *Am. J. Path.* **3**:251, 1927.

Under these conditions, it was thought to be of interest to continue and to extend these investigations in order to obtain, if possible, a confirmation of the former results; furthermore, we wished to determine whether inbreeding by successive matings of brother and sister rats, if still longer continued, would gradually increase the similarity between the individuality differentials of different litters within the same strain.

We were able to use, in these continued experiments, rats of strain A, as well as of strain B, which had been inbred by matings of brothers and sisters through from sixty to sixty-seven generations. First, however, we shall report on transplantations from rats belonging to strain A to rats belonging to strain B and vice versa. The results thus obtained will serve as a standard with which to compare the results of transplantations within the inbred strains A and B.

#### SERIES 1: TRANSPLANTATIONS OF TISSUES FROM STRAIN A TO STRAIN B OR VICE VERSA

Altogether 32 experiments in which tissues were transplanted from strain A to strain B or vice versa were carried out. In each case thyroid gland and cartilage and rats belonging to generations 60 to 66 were used for the transplantations. In the large majority of the experiments the specimens were examined between 19 and 25 days after the grafting, but in some cases they remained in the host for shorter or longer periods, in a few animals for as long as 60 and 100 days.

*Transplantation of Thyroid Gland.*—In a considerable number of animals the thyroid transplants were replaced by dense fibrous tissue in which were some remnants of blood pigment and collections of lymphocytes varying in size and density in different cases. This character was shown by the specimens examined after 60 and 100 days. In other rats remnants of thyroid acini were found in the periphery of the fibrous tissue. To a large extent these acini were without colloid and were surrounded by fibrous tissue which compressed them; but in other cases, in addition, some acini with colloid were preserved. However, as a rule, the number of the latter was restricted; and besides, the ring of these peripheral acini was not complete, and there was an excess formation of fibrous tissue surrounding them. Lymphocytes penetrated into the transplant and invaded and destroyed the acini. This lymphocytic infiltration was in some cases so intense that there resulted a picture almost resembling a lymph gland; also around the transplant as a whole, a dense lymphocytic infiltration could be noted, and wherever the infiltration was marked, the lymph vessels were stuffed with lymphocytes. Thus, in every instance, a great part of the thyroid transplant had been destroyed. There was also a partial destruction of the parathyroid due to infiltration with lymphocytes, and, furthermore, there was infiltration in the surrounding fat tissue.

*Transplantation of Cartilage, Fat, Bone, Bone Marrow and Striated Muscle Tissue.*—The cartilage transplant, in general, remained alive, especially if the piece of cartilage which was used for grafting was thin. Where it was thick, as it always is near the epiphyseal line, necrosis and solution often occurred in the center of the cartilage. Occasionally, however, owing to accidental conditions, such as injury during the operation or pressure exerted on the transplant later, there developed necrotic areas of variable length also in other parts of the cartilage. Frequently, but not in every case, islands of new cartilage were formed by the perichondrium around such necrotic areas, and this newly formed cartilage, usually consisting of small cells, penetrated into the necrotic cartilage and replaced it, at least in certain areas. Where the cartilage was thicker and where more intercellular hyaline substance was formed, the connective tissue and lymphocytes could readily push their way into it and partly destroy it. On the other hand, connective tissue and lymphocytes did not usually penetrate into cartilage consisting mainly of living cells. Furthermore, solution in the form of vacuoles in the intercellular substance of the cartilage occasionally could be seen.

In a number of cases we found the bone surrounded by a cellular tissue that evidently took its origin from the epiphyseal cartilage; from here the cells extended upward around the bone; at first a cellular cartilaginous tissue developed, which then gradually at a greater distance from the epiphyseal line changed into an osteoid tissue. The bone marrow was mostly replaced by loose or myxoid connective tissue, with or without addition of lymphocytes; other connective tissue penetrated into the bone marrow from the direction of the cut end of the bone; however, in certain places, there was still some necrotic bone marrow left, while only very rarely was any living marrow observed. Occasionally, connective tissue and lymphocytes penetrated even from the bone marrow into the epiphyseal columns of cartilage cells. In addition, in a number of specimens, remnants of striated muscle tissue, with chains of nuclei situated in the muscle fibers, which under these conditions were very thin, were visible, usually surrounded or invaded by much lymphocytic infiltration.

The fat tissue around the cartilage showed great variability as far as preservation was concerned. Almost always there was some increase in the connective tissue between the fat cells, and, in many cases, a great part of the fat tissue had been replaced by fibrous tissue; usually, however, some remnants of fat tissue were left between the strands of fibrous tissue. In some cases, especially in the specimens 60 or 100 days old, hardly any fat tissue remained around the cartilage. Not rarely we found in the remaining fat tissue the development of cystlike cavities due to the coalescence of adjacent fat cells. In addition, areas of necrosis were occasionally seen in the fat tissue usually adjoin-

ing necrotic areas in cartilage. As far as the lymphocytic infiltration in the fat tissue was concerned, it was either diffuse or localized especially around blood vessels in the fibrous bands traversing the fat. There usually were also some strands of lymphocytes in the fibrous tissue along the border of cartilage. However, in different cases the amount of lymphocytic infiltration varied greatly, and especially in specimens taken out at later periods, as, for instance, 60 or 100 days after transplantation, the lymphocytic infiltration was usually slight. On the other hand, in specimens removed at earlier periods it was generally marked, although, as stated, varying much in different transplants. Around the periosteum and the border between bone and cartilage there were also in some cases much fibrous tissue and a variable amount of lymphocytic infiltration.

#### SERIES 2: HOMOIOTRANSPLANTATION

We made two additional experiments in ordinary homoiotransplantation in noninbred rats, in order to compare the results with the results of the transplantations from strain A to strain B and vice versa. In these cases the examination took place twenty and twenty-two days after transplantation. Thyroid tissue was not preserved, only fibrous and fat tissue, with lymphocytic infiltration being found. On the other hand, as usual, the cartilage was, on the whole, well preserved, although the center, where the cartilage was thicker, was necrotic. Where the cartilage was thinner, some areas of necrosis were found, and plates of regenerated cartilage or connective tissue surrounded and grew into the necrotic cartilage and destroyed it. The columns of large bluish-staining cartilage cells were at least partly preserved near the border between bone and cartilage. The bone cells were mostly necrotic, but attached to the periosteum were areas that were alive. Again cartilage cells developed in the direction from the epiphyseal cartilage toward the bone, and the newly developed tissue surrounded the latter. At first the cartilage cells still possessed capsules, but farther away from the cartilage this tissue passed into an osteoid tissue. Bone marrow away from the cut side was necrotic, while nearer the cut end it was invaded by connective tissue. Around the cartilage there was fibrous tissue with some remnants of fat tissue and with masses of lymphocytes here and there. There was also some lymphocytic infiltration in fat tissue, and there was much fibrillar connective tissue around the cartilage. On the whole, no definite difference was noticeable between the results of transplantation of tissues from strain A to strain B or vice versa and the results of ordinary homoiotransplantation. We may then conclude that the transplantations from strain A to strain B and vice versa corresponded to homoiotransplantations. If we compare the results of these experiments with those obtained in our earlier

homoiotransplantations of thyroid and cartilage in the rat,<sup>3</sup> we find them, on the whole, similar. Considering separately the results in the experiments in which the examination of the thyroid grafts took place between the eighteenth and the thirtieth day, we find in twenty-six animals marked reactions, corresponding to the typical effects of homoiotransplantation. In five animals we obtained reactions intermediate between those seen after syngenesiotransplantation and those seen after homoiotransplantation, and in five animals effects typical of syngenesiotransplantation were found. When we transplanted cartilage and adjoining tissue we found in series 1 in twelve cases effects typical of homoiotransplantation or intermediate between those of homoiotransplantation and those of syngenesiotransplantation, while in three animals effects typical of syngenesiotransplantation were obtained. Later than thirty days, in a number of cases, a recovery of some transplants apparently took place; however, it is possible that a relationship between host and donor may have complicated the latter result. Transplantation from white into colored strains of rats led to still more decided reactions, corresponding in every case to very marked homoioresults.

### SERIES 3: TRANSPLANTATION WITHIN INBRED STRAINS

*Strain A.*—We shall now compare with the results obtained in transplantations from strain A to strain B and vice versa or in ordinary homoiotransplantations our more recent results in transplantations between different litters within the inbred strain A. We carried out 16 experiments of this kind. Thyroid gland and cartilage were used for grafting. In 8 experiments the animals belonged to the sixty-fifth, sixty-sixth and sixty-seventh generations of strain A; the transplants remained in the hosts for from 15 to 20 days. In the other 8 experiments generations 60 to 63 were used. In these cases the pieces remained in the hosts for periods varying from 25 to 100 days.

In general, the results in this series were not very different from those obtained when tissues were exchanged between strains A and B. Although we used animals inbred by means of successive matings of brothers and sisters through from 60 to 67 generations, no noticeable approach to a homozygous condition in the constitution of these animals had been accomplished. The results were therefore very different from those characteristic of autotransplantation. In only 5 of the 16 experiments were remnants of the thyroid gland found. In the rest of them merely fibrous tissue with more or less lymphocytic infiltration and some fat tissue remained from the thyroid transplant. In those in which thyroid tissue was preserved, two specimens were examined after

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3. Loeb, Leo: *Am. J. Path.* 2:302 and 315, 1926.

15 days, two after from 20 to 25 days and one after 100 days. But even in all of these the transplants differed very much from auto-transplants. In every case more or less thyroid tissue was destroyed, and many acini were without colloid and compressed; correspondingly there was usually much increase in connective tissue around the acini and around the whole of the transplant and the lymphocytic infiltration was marked, especially in places where well preserved acini were still present. In one case the specimen appeared so densely infiltrated with lymphocytes that the appearance almost of a lymph gland was produced. Frequently, the lymph vessels were stuffed with lymphocytes. In a specimen examined after 100 days, connective tissue with lymphocytes replaced a great deal of the thyroid transplant; there was much increase of connective tissue and in places much lymphocytic infiltration around and between the acini.

As to our results after the transplantation of cartilage, bone, muscle and fat tissue, they resembled very much those noted after transplantation of these tissues from strain A to strain B and vice versa. In general, the cartilage was well preserved, but there were in some specimens areas of necrosis, sometimes adjoining necrotic areas in fat tissue. While the fat tissue was usually not completely destroyed, it was always invaded and often more or less replaced by fibrous tissue. Also the number of lymphocytes was variable in different specimens, but usually the lymphocytic infiltration was much less intense than after transplantation of thyroid glands. In some specimens regeneration of perichondrial cartilage had taken place around necrotic cartilage; but parts of this regenerated cartilage could again become necrotic. While lymphocytes grew into necrotic cartilage in places, only exceptionally they penetrated into regenerated cartilage; for the most part they remained outside or penetrated only a little way into the perichondrium.

In the large majority of the cases bone marrow was replaced by fibrillar or myxoid connective tissue. The bone cells were generally necrotic, although some of them were occasionally preserved. Only in one case did we find some remnants of transplanted regenerated muscle tissue, in the form of chains of nuclei lying in thin muscle fibers. On the whole, the lymphocytic infiltration varied much in different cases and in different places; the relative proportion of fat tissue and of fibrous tissue replacing it varied also in different cases.

*Strain B.*—Within the inbred strain B, thirty-three experiments were carried out; in all cases thyroid gland and cartilage with adjoining tissues were transplanted, except in one instance in which ovary and uterus were used instead. The exchange of tissues was made between different litters of rats belonging to generations 60 to 66. In the large majority of cases, the examination took place between the twentieth and

the twenty-sixth day, but in two experiments it occurred after thirty and thirty-five days, respectively, in two experiments after forty days and in two experiments after 60 days.

In this series the results were better than those obtained in strain A; this becomes especially evident if we divide the experiments into two groups, the first one consisting of thirteen experiments, in which animals belonging to generations 64 to 67, and the second one consisting of twenty experiments, in which rats belonging to generations 60 to 63 were used. In the first group the results were decidedly better than those obtained after homoiotransplantation and after transplantation from strain A to strain B or vice versa. There were four experiments in which the results approached those of autotransplantation; this was true of the transplantation of thyroid gland, as well as of that of cartilage. In four other experiments the results were intermediate between those in syngenesiotransplantation and those in homoiotransplantation. In five experiments the results of marked homoiotransplantation were obtained; of the thyroid transplant, only fibrous tissue remained, and of the fat tissue around the cartilage, the greater parts had been replaced by connective tissue. Likewise, instead of bone marrow, loose connective tissue was found. In these cases, there was usually pronounced lymphocytic infiltration.

In the second group, there were two experiments in which the tissues were like autotransplants; in two or three other experiments the transplants resembled syngenesiotransplants, or they were midway between syngenesiotransplants and homoiotransplants. In the remaining fifteen experiments results characteristic of homoiotransplantation were obtained.

In general, then, there are noticeable in these experiments some beneficial effects from close inbreeding, but they are not pronounced. In the later generations the results are better than in generations 60 to 63. However, this may be due to a coincidence, because it is not probable that on reaching the sixty-fourth generation a sudden change occurred that led to a greater homogeneity of the individuality differentials. It is more probable that in the later generations the litters between which an exchange of tissues took place happened to be nearer related to each other, having split off from their common ancestors only at a relatively recent date, while in earlier generations they had presumably separated for a longer period.

In those cases in which grafts resembled autotransplants, we found a well preserved ring of acini, with good colloid, surrounding a relatively small amount of central connective tissue. Cartilage and fat tissue were well preserved, while bone marrow was usually at least partly preserved. In some cases, however, owing to accidental conditions, part of the cartilage was necrotic. Lymphocytic infiltration was lacking, and the



columns of cartilage cells near the bone were usually well preserved. In other instances there was associated with such results a slight, but distinct, lymphocytic infiltration, as an indication of a slight incompatibility between the differentials of host and transplant. Where reactions typical of homoiotransplantation were obtained, the thyroid tissue was as a rule entirely replaced by fibrous tissue, in which there were visible lymphocytic infiltration and remnants of blood pigment or, in other cases, remnants of acini lying at the periphery of dense fibrous tissue; in addition, fibrous tissue enclosed as a capsule and invaded the remnants of thyroid gland, and at the same time in some cases there was noticeable pronounced lymphocytic infiltration. In the majority of these acini the colloid had been lost, and as the result of pressure the acini were gradually destroyed. Around the cartilage the fat tissue had to a large extent been replaced by connective tissue, containing variable amounts of lymphocytes. There was a layer of lymphocytes, more or less complete, also around the cartilage. The bone marrow was fibrillar.

Conditions intermediate between these extremes of grafts resembling autotransplants and homoiotransplants were also obtained. In such an intermediate process, corresponding to the kind typically seen in syngenesiotransplants, we found; for instance, twenty days after transplantation, a ring of acini with preserved colloid; there were dense masses of lymphocytes in the periphery of the transplant and also in the adjoining fat tissue. Likewise the parathyroid tissue was well preserved, and there were numerous mitoses in parathyroid cells; at the same time we found masses of lymphocytes in such a specimen, but no increase in connective tissue. Cartilage and surrounding fat tissue were thus, on the whole, well preserved in these transplants.

If we compare our results of transplantations within the inbred strains A and B with those obtained in transplantations from A to B or from B to A or in ordinary homoiotransplantation, we find on the whole the results in the former two series slightly better than those in the latter. However, the number of transplantations in strain B in which results corresponding to autotransplantation were obtained is greater than may be expected in ordinary homoiotransplantation, but the average grade in these two series is only slightly higher than the grade found in homoiotransplantations.

It is also of interest to compare the results obtained in inbred strain B with those in syngenesiotransplantation in not closely inbred families, although father and mother may have belonged to families that were related.<sup>4</sup> In strain B, in the generations from the sixty-fourth to the

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4. Loeb, Leo: *Am. J. Path.* **3**:45, 1927.

sixty-seventh, the results were: auto, 4 cases; syngenesiohomoio, 4 cases; homoio, 5 cases. In the generations from the sixtieth to the sixty-third, the results were: auto, 2 cases; syngenesio or syngenesiohomoio, from 2 to 3 cases; homoio, 15 cases. In ordinary syngenesiotransplantation, the results were as follows: auto or approaching auto, 25 cases; good syngenesio, 8 cases; syngenesio, 6 cases; syngenesiohomoio, 8 cases; homoio, 7 cases. If we consider separately the earlier cases, examined between nineteen and twenty-five days following transplantation, the results are still better: auto, 10 cases; syngenesio, 1 case; syngenesiohomoio, 2. If we group together those specimens showing results typical of autogenous and syngenesiotransplantation (group 1), and the specimens showing results characteristic of homoiotransplantation and results intermediate between syngenesiotransplantation and homoiotransplantation (group 2), we find in ordinary syngenesiotransplantation thirty-four belonging to group 1 and fifteen belonging to group 2. While in strain B about eight belong to group 1 and twenty-five to group 2.

There is thus a marked difference between the results obtained in group B and in ordinary syngenesiotransplantation. It is possible that in the transplantations in strain B in which better results were obtained the two litters to which host and donor respectively belonged were more nearly related to each other than the average litters, and that in the majority of cases the two litters had been split off from the common ancestor a considerable number of generations back. Yet, even if the splitting off occurred as early as after thirty generations of close inbreeding by successive matings of brothers and sisters, under ordinary circumstances we should have expected an almost complete homozygotic condition to exist at that time. Further separate propagation by brother and sister mating of two litters could have led to such unfavorable results, as far as an approach to a homozygous condition is concerned, only if we assume either that, as a result of selective mating in each successive generation, a homozygous condition had not yet been reached at the time when the splitting off occurred, or that the occurrence of mutations prevented the approach to a homozygous state.

*Summary.*—Our present results, then, confirm in all essential respects our former conclusions. Exchange of tissues between different litters of strain A and of strain B does on the average give results only very slightly better than those obtained in homoiotransplantation, and again we must point out the difference noted in inbred rats and guinea-pigs. In the latter the homozygous condition attained through continued mating of brother and sister generation after generation was much more definite, and the results of transplantation within the inbred families were much better, than we found in the inbred rats.

SERIES 4: EXCHANGE OF TISSUES BETWEEN DIFFERENT LITTERS  
OF THE GROUP  $(A \times B) F_4$ 

Through mating of rats belonging to strain A with rats belonging to strain B a generation of hybrids,  $(A \times B) F_1$ , was produced. These were then propagated through three or four generations of successive matings of brothers and sisters, so that  $(A \times B) F_4$  and  $F_5$  were obtained. Transplantations were then carried out from one litter to another litter of this kind, usually the animals belonging to generation  $F_4$  being used; only in one case exchange of tissues took place between rats belonging to generation  $F_5$ . Altogether fourteen experiments were carried out in this series. In two of these ovaries and uterus, in the remaining ones, thyroid gland and cartilage, were transplanted. In two experiments in which ovaries and uterus were used, the examination took place after two and ten days, which represent too early a period for an exact grading of the results, as the full reaction had not yet developed at this period. However, already at this time definite lymphocytic reactions were noticeable in the transplants, and in one case there were, in addition, much necrosis and connective tissue in the graft. In the twelve remaining experiments, the examination took place between the twentieth and the twenty-third day. In four of these experiments reactions approximately intermediate between those observed after syngenesiotransplantation and those after homoiotransplantation were obtained. Certain parts of the thyroid gland were preserved, but they were markedly invaded by lymphocytes and were thus in process of destruction; in addition, there was observed an ingrowth of connective tissue into the transplant. The cartilage was usually well preserved, but here also more or less lymphocytic infiltration was found, and also in the fat tissue there was an increase of connective tissue and of lymphocytic infiltration. On the outside of the bone a formation of new cartilaginous tissue that gradually passed into osteoid tissue could be seen. The bone marrow was still in some places partly preserved and in other places was replaced by connective tissue. In the other eight specimens, marked homoioreactions were obtained; the thyroid glands usually were completely destroyed, and the fat tissue to a great extent was replaced by fibrous tissue. The bone marrow was replaced by connective tissue or was necrotic. However, in some cases regeneration of cartilage was noted in places where the cartilage had been wounded, or around necrotic cartilage; thus the necrotic material could be invaded and replaced by regenerating cartilage and, in addition, nodules of regenerating cartilage formed. In four experiments syngenesiohomoioreactions and in eight marked homoioreactions were obtained. On the whole, the results in this series were therefore unfavorable and not much better than those seen in homoiotransplantation.

SERIES 5: TRANSPLANTATION FROM  $(A \times B)$   $F_4$  TO B OR A AND  
THE RECIPROCAL TRANSPLANTATION FROM B OR A TO  
 $(A \times B)$   $F_4$

In this series thyroid gland and cartilage were transplanted either from a hybrid between strain A and strain B to one of the parent strains, after these hybrids had been inbred for four generations by means of successive matings of brothers and sisters, or the reverse transplantation was carried out from one of the parent strains to the hybrid. Such experiments were thought to be of interest, because in the work with inbred strains of guinea-pigs a striking difference was observed between the results in these two types of transplantation; here transplantation from one of the parent strains to the hybrid strain gave excellent results approaching those observed after autotransplantation, while the reciprocal transplantation was relatively unfavorable. In this series we could not expect to find a difference corresponding to those noted in inbred families of guinea-pigs, because neither the individuals of strain A nor those of strain B had attained a very definite homozygous condition, as we have shown in this paper.

*Transplantations from Parent Strain to Hybrid.*—Thirteen experiments in transplantation from the parent strain to the hybrid were carried out. In nine cases, in which the examination took place between sixteen and thirty-seven days after transplantation, homoioreactions were obtained. However, in four of these cases, the results were only of an approximate character, an exact classification not being possible, because only cartilage without a sufficient amount of other tissue was recovered at the conclusion of the experiments. In two cases, examined after twenty-nine and fifty days, the results were intermediate between those of syngenesiotransplantation and those of homoiotransplantation. In an additional case the result corresponded to that of syngenesiotransplantation, and here the examination took place after thirty-seven days. In the last case, examined after twenty days, the pieces resembled autotransplants.

*Transplantation from Hybrid to Parent Strains.*—In the reciprocal transplantations somewhat more unfavorable results were obtained. In eleven of thirteen experiments approximate homoioreactions were found; in one case the result was intermediate between a typical homoioreaction and a syngenesioreaction, and in the last case it corresponded to a syngenesioreaction.

*Summary.*—The difference between the results in these two series of transplantations is so slight that we cannot attach much significance to it. We may summarize our results as follows: (a) In transplantation from parent to hybrid, the 13 experiments carried out yielded 9 homoioreactions, 2 syngenesiohomoioreactions, 1 syngenesioreaction and

1 autoreaction. (b) In transplantation from hybrid to parent, the 13 experiments carried out gave 11 homoioreactions, 1 syngenesiohomoioreaction and 1 syngenesioreaction.

SERIES 6: EXCHANGE OF TISSUES BETWEEN BROTHERS WITHIN  
INBRED STRAINS

*Transplantation of Thyroid Gland and Cartilage or of Ovary and Uterus from Brother to Brother (or from Sister to Sister) in Inbred Strain A.*—Seventeen experiments were carried out; in thirteen thyroid gland and cartilage, and in four uterus and ovary were used for transplantation. In six experiments the animals belonged to the sixty-sixth and sixty-seventh inbred generation, while in the remaining eleven experiments they belonged to generations 60 to 63. The pieces were removed for examination between the twentieth and the fiftieth day following transplantation. In three of the six experiments in which the sixty-sixth and sixty-seventh generations were used, thyroid gland and cartilage had the character of autotransplants; one of these was examined after forty-two days and two after twenty days. In the cartilage transplants, fat tissue, columns of large cartilage cells at the border between bone and cartilage and regenerating muscle tissue were well preserved. The condition of the bone marrow varied; connective tissue replaced the bone marrow partly or even entirely in some of these cases.

In the three remaining cases of this group results characteristic of syngenesiotransplantation were obtained. In two of these cases—in the third one the thyroid transplant was not found—the structure of the thyroid and parathyroid transplants corresponded to the structure of autotransplants, but subsequently masses of lymphocytes penetrated into the graft and destroyed it in part. In the cartilage-fat-bone-muscle transplant the preservation was, on the whole, also very good, but there was some invasion by lymphocytes which in these specimens was, however, less marked than in the thyroid. These cells destroyed some of the muscle tissue, but the bone marrow was partly preserved. The intensity of lymphocytic infiltration varied in different cases, in two of which the examination took place fifty days, and in the last one twenty-five days, after transplantation.

In seven experiments with animals of generations 60 to 63, thyroid gland and cartilage were transplanted. In four of these cases, examined between twenty and thirty-five days following transplantation, results approaching, but not entirely identical with, those of autotransplantation were obtained; after thirty and thirty-five days the lymphocytic infiltration began to be a little more marked in places, although otherwise the results were very good, and even some mitoses were found in the transplanted parathyroid cells; in an additional animal the condition

was intermediate between autoreaction and syngenesioreaction. In two other specimens examined after twenty-five and forty-five days, respectively, a pronounced syngenesioreaction was observed. In these two cases, the lymphocytic infiltration was intense, and the thyroid gland in one specimen had the appearance almost of a lymph gland. While there was hardly any connective tissue reaction, the dense masses of lymphocytes had destroyed a good part of the thyroid, and likewise a part of the parathyroid. Again in the fat tissue the lymphocytic infiltration was less marked than in thyroid and parathyroid tissue; furthermore, there was no increase of connective tissue in the fat tissue. The bone marrow was partly preserved.

In four rats into which pieces of ovary and uterus had been transplanted, the examination took place between twenty and thirty days after transplantation. Parts of uterus and ovary were preserved, but there was everywhere more or less marked lymphocytic infiltration, and results characteristic of autotransplantation were not observed; however, an exact gradation in several of these cases was not possible.

Altogether, therefore, in eight cases results characteristic of or approaching autotransplantation were obtained, and in five cases the pieces resembled syngenesiotransplants. While in the four experiments in which pieces of ovary and uterus were transplanted, an exact gradation was not possible, we can be certain that in none of them was the effect of autotransplantation obtained. On the whole, the results in transplantation of ovary and uterus corresponded, therefore, to those found in syngenesiotransplantation, although one or two of them may have approached the range characteristic of homoiotransplantation.

*Transplantation of Tissues from Brother to Brother in Inbred Strain B.*—In nineteen experiments tissues were transplanted from brother to brother within strain B. The pieces were removed from the hosts for examination at various times between twenty and sixty days after transplantation. In thirteen experiments—in some of which the transplants were examined as late as sixty days after grafting—the results corresponded to or approached those of autotransplantation. In one of these cases the thyroid and parathyroid grafts resembled autotransplants, whereas the cartilage transplant showed a slight, but distinct, increase in connective tissue and a slight lymphocytic infiltration, although, on the whole, cartilage, fat tissue and muscle tissue were well preserved. In two cases, pieces were transplanted from one brother or sister into two other brothers or sisters. In one of these cases, the grafts in both hosts behaved, after sixty days, like autotransplants; in the second case, in one host, the results in the thyroid graft were like those of autotransplantation, while those in the cartilage graft corresponded to the results of syngenesiotransplantation, and in the other host the results were those of homoiotransplantation.

In three specimens the examination of which took place twenty days following the operation and in one of which ovary and uterus were transplanted instead of thyroid tissue and cartilage, the results of syngenesiotransplantation were obtained. There were two specimens to which we have referred which showed a rather mild homoioreaction and one specimen in which the condition was intermediate between homoioreaction and syngenesioreaction.

In the instances in which results approaching those of autotransplantation were obtained, bone marrow was well preserved as late as sixty days following the grafting, and, as usual, the bone spicules in the bone marrow were much better preserved than the cortical bone proper. Grafts in which we found a localized lymphocytic infiltration that exceeded in intensity the very slight collections of lymphocytes occasionally observed in an autotransplant, but in which the connective tissue was not increased, were classed as approaching autotransplants. In accordance with our previous observations in noninbred strains, it was to be expected that in the course of time this lymphocytic infiltration would increase in intensity and exert a destructive effect on the transplant.

In the specimens corresponding to homoiotransplants, the bone marrow, as usual, was necrotic or replaced by connective tissue, while in those corresponding to syngenesiotransplants parts of the bone marrow were preserved; in certain places osteoid tissue was produced which in the periphery seemed to undergo transformation into myxoid connective tissue. In grafts that behaved like syngenesiotransplants, there was a dense lymphocytic infiltration in the thyroid and in the parathyroid, while in the fat and muscle tissue, it was somewhat less marked; in addition, there was found in the cartilage specimen some increase in connective tissue.

If we compare the results in these experiments with those in the corresponding experiments in our earlier investigations in inbred rats, in which transplantations of tissue from brother to brother were made, we find that the results in our present series are somewhat better. They are likewise better than the results of Loeb in noninbred rats in ordinary transplantations of tissue from brother to brother.<sup>4</sup> On the other hand, they are distinctly less good than the results obtained by Loeb and Wright<sup>2</sup> in inbred guinea-pigs in transplantations from brother to brother. It is probable, therefore, that slight progress toward a more homozygous condition in the rats has been accomplished through the continued inbreeding; however, there can be no doubt that the reactions between host and transplant are more marked in rats inbred through more than sixty generations by matings of brothers to sisters than the reactions in guinea-pigs inbred through approximately twenty generations.

*Summary.*—The results of transplantation of tissues from brother to brother in the inbred strains may be summarized as follows: 1. In strain A, autoreactions or autosyngenesioreactions were obtained in eight experiments and syngenesioreactions in five experiments. In four experiments an exact grading was more difficult, but we can be certain that the results did not correspond to autoreactions. 2. In strain B, autoreactions were obtained in thirteen experiments and syngenesioreactions in three experiments, a syngenesiohomoioreaction was found in one case and mild homoioreactions were found in two cases. In both series combined we found autoreactions or autosyngenesioreactions twenty-one times, syngenesioreactions eight times, mild homoioreactions twice and a syngenesiohomoioreaction once. In four cases the grading was uncertain, but the results did not correspond to those of autotransplantation.

*Multiple Simultaneous Transplantations in Inbred Strain B.*—In the sixty-seventh generation of strain B, four pieces of thyroid gland and two pieces of cartilage from two brothers were grafted into a third brother. All four pieces of thyroid gland examined thirty-two days following transplantation behaved like autotransplants. Well preserved ducts with squamous epithelium were found in the center of the grafted tissue; only an occasional, very small nest of lymphocytes was seen in the connective tissue; a few isolated lymphocytes were noted, but not in every specimen, around some acini or around the parathyroid. Cartilage and fat tissue appeared normal; there were here no collections of lymphocytes, nor any noticeable increase in connective tissue. These results agree with the previous ones of Loeb, so far as they show that a deficit in function of the thyroid gland in the host is not required for the successful transplantation of this organ.

*Serial Transplantation in Inbred Strain B.*—We transplanted two pieces of thyroid gland from a rat of strain B to his brother. Twenty-five days later both transplants were removed and retransplanted to a sister. Twenty-five days following the second transplantation, one of the grafts was removed from the animal for microscopic examination. This piece had therefore been transplanted twice in succession and each time had remained twenty-five days in the host; altogether it had been in brother and sister for a period of fifty days (twenty-five plus twenty-five days). The second transplant was retransplanted again to a sister of the bearer and examined twenty-three days following the last transplantation (twenty-five plus twenty-five plus twenty-three days equaling seventy-three days).

*Transplant Examined After Fifty Days (Twenty-Five Plus Twenty-Five Days):* The transplanted thyroid, as well as the transplanted parathyroid and fat tissue, was well preserved; all behaved like autotransplants. There was a limited amount of loose connective tissue in



the center of the graft, and the acini were in close contact with each other. Only a few lymphocytes were found in one place.

Transplant Examined After Seventy-Three Days (Twenty-Five Plus Twenty-Five Plus Twenty-Three Days): The acini of the thyroid, containing solid colloid, as well as the parathyroid, were well preserved, but in the connective tissue traversing the fat, which surrounded the thyroid graft, there was a diffuse lymphocytic infiltration, extending even into the region of the acini. In places the lymphocytes penetrated still deeper between the acini and also a short distance into the parathyroid on the other side, and some had invaded the surrounding muscle tissue of the host; the lymph vessels were studded with these cells. There was no increase in connective tissue.

We have to deal in this case with a syngenesioreaction and not, as in the first transplant, after twenty-five days plus twenty-five days, with an autoreaction. While, therefore, this experiment confirms our conclusions that the individuality differentials have not yet become identical in brothers in inbred rats, the differentials are sufficiently similar to make possible the serial transplantation of thyroid, a task that could not be accomplished in the case of a typical homoiogenous relationship between host and donor. This experiment confirms a result obtained formerly in transplantation in inbred guinea-pigs.<sup>2</sup>

#### SERIES 7 TRANSPLANTATION FROM BROTHER TO BROTHER (OR FROM SISTER TO SISTER) IN ( $A \times B$ ) $F_1$ STRAIN

It was of interest to compare with the brother to brother transplantations in inbred strains A or B brother to brother transplantations in hybrids ( $A \times B$ )  $F_1$ . Theoretically, it should be expected that, although inbreeding had taken place through several generations, the genetic constitutions and therefore the individuality differentials of brothers or sisters in such litters would be less similar to each other than the individuality differentials of brothers in inbred strains A or B, a conclusion which was confirmed by our investigations.

We carried out thirty experiments; however, two of these must be discarded on account of the possibility that a mistake was made in the labeling of the specimens. Of the remaining twenty-eight, two were examined after ten and twelve days, respectively, which represent, perhaps, too short a period for an accurate grading. In nine additional experiments ovary and uterus were transplanted, and in these specimens an accurate grading is likewise difficult. But, if we make an approximate classification of all but two specimens, we obtain the following results: In six cases the grafts corresponded to syngenesiotransplants; in eleven cases their condition corresponded to a condition intermediate between that of syngenesiotransplants and that of homoiotransplants, and in nine cases the typical results of homoiotransplantation were

obtained. Of the two doubtful transplants, one appeared like an autotransplant and the other one like a homoiotransplant. If we except, therefore, the result obtained in one experiment, in none of these cases did we find grafts that resembled autotransplants. These results are therefore much inferior to those noted in transplants between brothers within the inbred strains A or B; they are inferior even to those found in ordinary syngenesiotransplantation. The long-continued separate breeding of the A and B strains apparently led to a marked difference in the average of the individuality differentials in these two strains, a difference which the short period of inbreeding in hybrids between A and B could not overcome.

In cases of syngenesioreaction we found as usual small masses of lymphocytes around and between certain acini, and, also, in the parathyroid there was some increase in lymphocytes in the lymph vessels. Bone marrow could be preserved in such transplants; likewise the fat tissue was well preserved, showing merely a moderate increase in the thickness of the connective tissue septums. Between the regenerating muscle fibers some lymphocytic infiltration was noted. It was also of interest that near wounds of the cartilage, tissue formed, which resembled connective tissue in structure, but which in reality represented growing perichondrial or cartilage cells. In the ovarian transplants of this series we also observed usually more or less marked lymphocytic infiltration, and in the uterine grafts we found, as a rule, in the areas of transition between cellular fibroblastic connective tissue and necrotic material, processes of hyalinization, indicating insufficient nourishment of the grafted tissue. In a specimen in which a syngenesioreaction was obtained, we noted that cartilage cells proliferated not only toward the outside of the bone, but also into the bone marrow spaces, which thus became filled with cartilage. Furthermore, tissue apparently transitional between cartilage and osteoid tissue was found.

Of special interest was a transplant of cartilage examined after twenty-five days, which behaved like a syngenesiotransplant, but in which proliferating perichondrial cells, during the process of transformation into cartilage cells, showed an active mitotic proliferation. Even the young cartilage cells that developed from the perichondrial tissue showed mitoses, but mitotic activity ceased as soon as vacuoles appeared. There was at the same time a marked lymphocytic infiltration around the transplanted living cartilage, and lymphocytes penetrated even into the perichondrium.

#### SUMMARY AND CONCLUSIONS

In these experiments we used tissues from rats of strains A and B, inbred through from sixty to sixty-seven generations by matings of brother and sister rats. The results of transplantation of tissues from

strain A to strain B corresponded to the results of ordinary homoio-transplantation. Transplantation between different litters within inbred strain A likewise gave results very similar to those obtained in homoio-transplantation, and this was true of grafts of thyroid gland, as well as of those of cartilage.

Transplantation between different litters within inbred strain B gave better results, but even these results remained far behind those obtained in ordinary syngenesiotransplantation from brother to brother. They were between the typical results of homoiotransplantation and those of syngenesiotransplantation, but nearer the former than the latter. As was to be expected, transplantation to different litters in group  $(A \times B) F_4$  or  $F_5$  led to marked homioireactions. Transplantation from  $(A \times B) F_4$  to one of the parent strains or vice versa gave results corresponding to or approaching homioireactions; but in accordance with expectation the results were somewhat better in transplantation from parent to hybrids than in the reciprocal transplantation. However, it is doubtful whether this difference is sufficiently great to be of significance.

In brother to brother transplantation within inbred strains the results obtained were somewhat better than those in our earlier experiments in inbred rats, and in strain B they were perhaps slightly better than in strain A. The reactions which we found were somewhat less marked than those which we observed in ordinary syngenesiotransplantation, but the differences observed between these two series were, after all, not striking. The following summary allows a comparison between these two series: 1. Ordinary syngenesiotransplantation: autoreactions or autosyngenesioreactions, 33; syngenesioreactions, 6; syngenesiohomoio-reactions, 8; homioireactions, 7. 2. Transplantation within inbred strains: autoreactions or autosyngenesioreactions, 21; syngenesioreactions, 8; syngenesiohomoio-reactions, 1; mild homioireactions, 2; uncertain, but not autoreactions, 4.

Multiple simultaneous transplantations in brothers of inbred strain B, under conditions of approximate identity of the individuality differentials of hosts and donor, confirm our former conclusion that a deficit in internal secretion of the thyroid gland is not necessary for the preservation of the thyroid transplant. Two and even three successive transplantations of thyroid and parathyroid succeeded in brothers and sisters of strain B; however, in the third transplantation a syngenesio-reaction appeared, indicating that in this case also a complete identity of individuality differentials between brothers and sisters had not yet been obtained. In general, we must again state in this connection that there is no certainty that when a certain graft in a host behaves like an autotransplant at a certain time following transplantation, it will continue to behave in this way; on the contrary, there are many indications that at a still later period a reaction on the part of the host may set in.

Much more unfavorable results than in transplantation within the same litter in strains A and B were obtained in brother to brother transplantation in  $(A \times B) F_4$  hybrids. On the other hand, the results here were somewhat better than in ordinary homoiotransplantation. In ordinary homoiotransplantation the results were 5 syngenesioreactions; 5 syngenesiohomoioreactions; 26 homoioreactions. In transplantation between brothers of  $(A \times B) F_4$  the results were: 6 syngenesioreactions; 11 syngenesiohomoioreactions; 9 homoioreactions. No specimens resembling autotransplants were obtained, because, in combinations between the gene sets of A and B, the probability that identity between the individuality differentials has developed is not great.

On the whole, we may therefore conclude that notwithstanding continued inbreeding in strain A and strain B a condition approaching a homozygous genetic constitution within the same strain has not been attained. On the contrary, our results indicate much more marked heterozygous constitutions after inbreeding in rats through from sixty to seventy generations than Loeb and Wright found in guinea-pigs after inbreeding of these through a much smaller number of generations. There is, however, some indication that a slight advance toward greater similarity of the individuality differentials within the same strain has been made since our first transplantations, in which we used animals belonging to generations 30 to 47. As to the factors underlying these results we must consider, as we did formerly, (1) the selection of heterozygous individuals during the process of close inbreeding; (2) the possibility that a splitting off of litters and separate propagation within the strains A and B may play a certain rôle, but such a splitting off could lead to the maintenance of a heterozygous condition only in case some other factor had prevented the reaching of a homozygous constitution at the time when the separate breeding of the two litters set in; (3) the occurrence of mutations must be considered as a possible factor. While at present it is impossible to determine what rôle mutations play in the results that we obtained, there can be little doubt that the selection of the strongest individuals during the process of inbreeding has been of great significance in the persistence of the heterozygous constitution of the inbred rats, as revealed by the results of our transplantations.

# CHRONIC BRONCHITIS WITH FOREIGN BODY (ELASTIC FIBERS) REACTIONS IN THE LUNGS \*

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Many substances originating from tissues or their fluids become insoluble because of necrosis or chemical changes, and the inert material, as an irritant, stimulates a mass of chronic granulation tissue, and this is commonly designated "a foreign body tissue reaction." Noteworthy among the cellular constituents in this reaction are the foreign body giant cells. Cholesterol,<sup>1</sup> lipins and their derivatives,<sup>2</sup> salts of calcium and of iron<sup>3</sup> and incrustated connective tissue fibers<sup>4</sup> are tissue derivatives that become insoluble and act as foreign substances. Elastic fibers of the connective tissues have been reported to be the substance stimulating foreign body inflammatory reactions in many parts of the body,<sup>5</sup> but rarely have these lesions been found in the tissues of the lungs. Kockel<sup>6</sup> seems to have been the first to record such a reaction in the lungs.

A man, 33 years of age, died of pernicious anemia. Lesions in the walls of blood vessels of the lungs showed giant cells with inclusions like elastic fibers, although the fibers failed to react with specific stains. Similar fibers in the alveolar and arterial walls reacted variably with the tests for elastin, iron and calcium.

The following year Davidsohn<sup>7</sup> reported a study of lungs; one from a man, 27 years old, who died with sarcoma of the sacral vertebrae, a second from a man, aged 22, who had carcinoma of the stomach with metastases to the vertebral column, pelvis and humerus, and a third and fourth, from Virchow's museum, for which there were no clinical records. There was a pronounced fragmentation of elastic fibers in the alveolar septums and deposits of calcium in the septums, in the interstitium and in the walls of arteries, but no giant

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\* From the Henry Baird Favill Laboratory of St. Luke's Hospital.

\* Read before the Chicago Pathological Society, Jan. 12, 1931.

1. LeCount, E. R.: Tr. Chicago Path. Soc. **5**:53, 1902.

2. Mallory, F. B.: Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1914, p. 52.

3. Haythorn, S. R.: Multinucleated Giant Cells, Arch. Path. **7**:651, 1929.

4. Hektoen, L.: Tr. Chicago Path. Soc. **5**:51, 1902.

5. Hirsch, E. F., and D'Andrea, D.: Iron Incrustated Fiber Inclusions in Giant Cells, Arch. Path. **8**:628, 1929.

6. Kockel, R.: Arch. f. klin. Med. **64**:332, 1899.

7. Davidsohn, C.: Virchows Arch. f. path. Anat. **160**:538, 1900.

cell reaction. Bittrolff<sup>8</sup> observed inflammatory changes against inert tissue fibers in the lungs of a woman, aged 25, who clinically had organic decompensation of the heart and chronic passive hyperemia. Giant cells with inclusions were found in and about the walls of the larger blood vessels. The inclusions were concentrically laminated, oval or round structures, and slender fibers. These gave the specific reactions with various stains for elastic tissue and also the microchemical reactions for calcium and iron. Bittrolff concluded that they were elastic fibers undergoing secondary changes and incrustated with iron and calcium. In 1912, Schum<sup>9</sup> studied the lung of a woman, aged 47, who, according to the clinical diagnosis, died of asthma, nephritis and myocarditis. There was a generalized venous stasis. Histologically the lung had many foreign body giant cells distributed about the bronchi and arteries, where the elastic fibers (according to various stains) had undergone degenerative changes. The fibrillar and concentrically laminated inclusions of the giant cells reacted somewhat to stains for elastin and to the microchemical tests for iron. Only traces of calcium were present. A year later Gigon<sup>10</sup> found concentrically laminated fibrillar structures incrustated with iron and calcium in the walls of the medium and small blood vessels in the lungs of a man, 31 years old, who, according to the clinical diagnosis, had an infarct of the lung and chronic passive hyperemia. He saw no giant cells. Recently Dugge<sup>11</sup> reported lesions in the lungs of a man, 30 years old, who for many years had had asthma and whose death was due to chronic bronchitis and bronchopneumonia. The fibrous tissue about the bronchi contained foreign body giant cells with inclusions of fatty acid crystals, cholesterol and concentrically laminated amorphous bodies. The latter, he concluded, were elastic fibers incrustated with iron and calcium.

A white man, a blacksmith, aged 58, was in the care of Dr. C. F. G. Brown at St. Luke's Hospital for a short time in 1925 because of a sprain of the back and glycosuria. Medical management of the diabetes and treatment for several minor injuries and infections kept him in fair health until Jan. 19, 1930. Then he again entered the hospital because of an infection of the left foot. This was drained surgically. He had a septic fever and many reactions to insulin. The number of erythrocytes diminished from 4,000,000 at the time of admission to 2,580,000 per cubic millimeter on March 29; the hemoglobin decreased from 80 to 40 per cent. On April 27, following reaction to insulin, the patient died.

The postmortem examination was made sixteen hours after death by Edwin F. Hirsch. The essential observations were: diabetes mellitus; bilateral bronchopneumonia; abscesses of the lungs; chronic bronchitis; marked emphysema, hyperemia and edema of the lungs; cloudy swelling of the myocardium, kidneys and

8. Bittrolff, R.: *Beitr. z. path. Anat. u. z. allg. Path.* **49**:213, 1910.

9. Schum, H.: *Virchows Arch. f. path. Anat.* **208**:446, 1912.

10. Gigon, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **55**:46, 1913.

11. Dugge, W.: *Virchows Arch. f. path. Anat.* **277**:757, 1930.

liver; moist gangrene and chronic osteomyelitis of the left foot and ankle; left serofibrinous pleuritis; recent hemorrhage beneath the left parietal pleura; surgically incised abscesses of the left axilla; osteomyelitis of the right tenth rib; marked senile arteriosclerosis of the aorta and its main branches; right chronic fibrous pleuritis, etc. The body weighed 107½ pounds (48.7 Kg.) and was 172 cm. long. There was about 25 cc. of serofibrinous exudate in the left pleural cavity. The pleura of the left lung was hyperemic and posteriorly covered with fibrin. Opposite the right tenth rib, 8 cm. from the spine, was a radiating scar of the parietal pleura 3 by 2.5 cm. in its maximum dimensions, and opposite this osteomyelitis of the rib. The right lung weighed 1,270 Gm.; the left, 1,265 Gm. Both were boggy, except in the marginal emphysematous regions. There was a marked subpleural edema of the right lung, and the pleura of the left was rough with plaques of fibrin. Beneath one of these, toward the lateral margin of the upper lobe, was an abscess, 2 cm. in diameter, filled with a yellow exudate. There were other nodules of the same size and content, one in the upper lobe of the left lung and two in the lower lobe of the right lung. Otherwise there was marked edema. The left upper lobe was similar. The left lower lobe was crepitant only in the upper lateral portions, and the remainder was studded with gray, granular, firm regions, from 0.2 to 1 cm. in diameter. A purulent material was expressed from the larger consolidated places and a yellow exudate from the smaller bronchi of both lungs. The lining of the pulmonary arteries had fatty changes. *Staphylococcus aureus* was isolated from the abscesses in the lungs.

The lungs microscopically were extensively consolidated by bronchopneumonic exudates and abscesses. They showed the changes usually present with chronic passive hyperemia and chronic emphysema. The unusual lesions were acute bronchial and peribronchial exudates associated with foreign body granulation tissues containing many giant cells (fig. 1). These giant cells were as many as 20 to 1.5 sq. mm., ranged between 0.04 and 0.2 mm. in diameter and occurred also in portions of lung tissue considerably removed from the pneumonic regions and abscesses. They contained coiled and sinuous fibrils (fig. 2) of connective tissue, and some were vacuolated. Similar fibers occurred in the lumens of the bronchi and alveoli. They reacted in general as do elastic fibers in sections stained with phosphotungstic acid-hematoxylin, van Gieson's stain, Weigert's elastic stain, Verhoeff's stain and Unna's orcein. The fiber inclusions of the cells did not react with the usual microchemical tests for iron and calcium. The general configuration and distribution of the many vacuoles in the giant cells suggested the presence of fats. However, none of the pulmonary tissues had been fixed in formaldehyde solution and specific stains for lipins could not be made.

According to Macklin,<sup>12</sup> the elastic tissue of the respiratory system is continuous from the larynx to the alveoli of the lungs, where it is distributed as delicate strands in the septums. In the bronchial walls the widest layer courses longitudinally in the mucosa, branches with the air tubes, and becomes thinner as the bronchi decrease in size. Outside of this band is a smaller band, distributed transversely and interlacing with the muscle of the wall. Other aggregates of elastic tissue are present in the larger tubes and course obliquely. The changes

12. Macklin, C. C.: Functional Aspects of Bronchial Muscle and Elastic Tissue, Arch. Surg. 19:1212, 1929.



Fig. 1.—Inflammatory exudate, elastic fibers and foreign body giant cells in and about a bronchiole;  $\times 160$ .



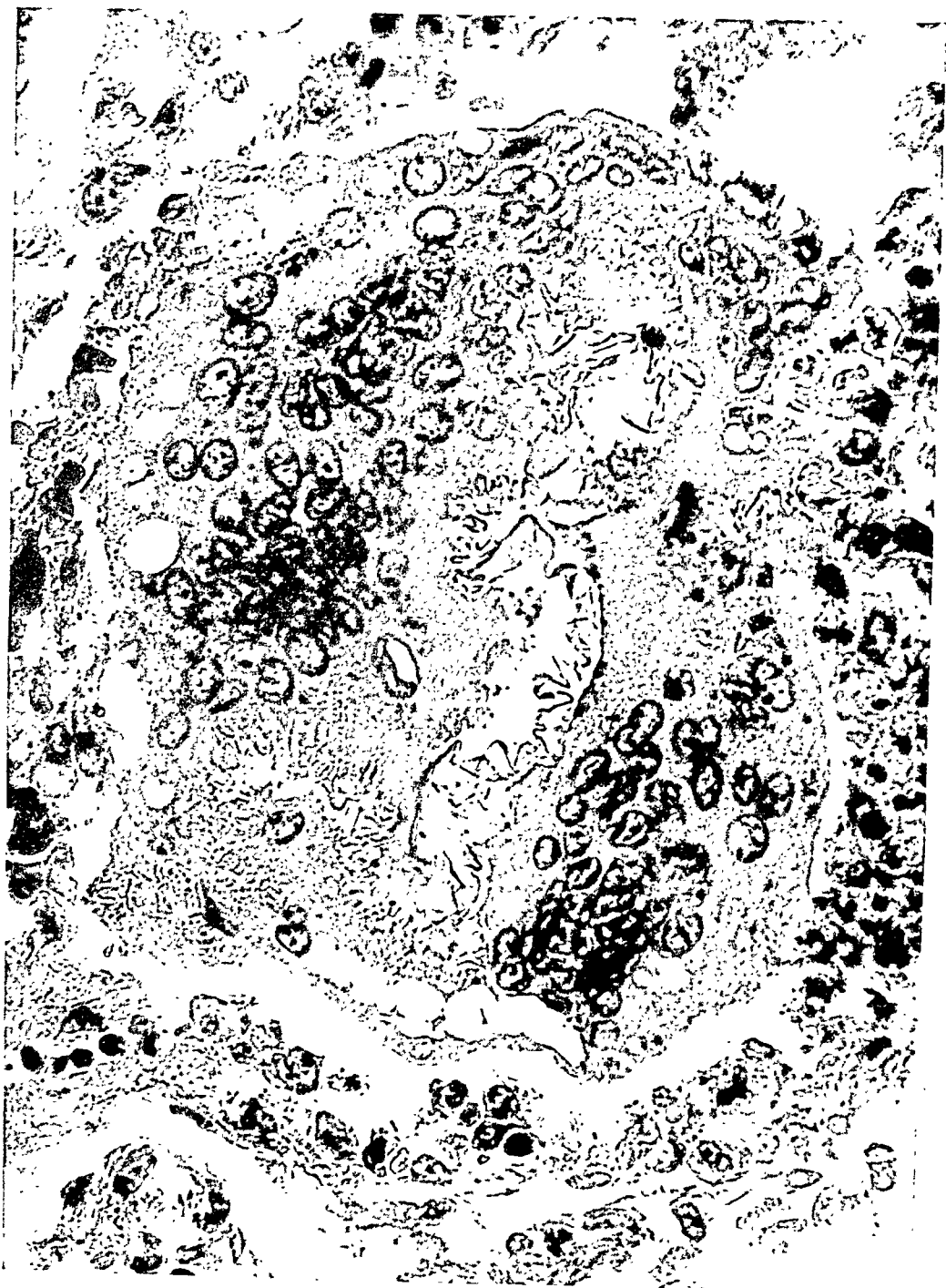


Fig. 2.—Photomicrograph of a giant cell with elastic fiber inclusions and vacuoles;  $\times 630$ .

whereby some of these elastic fibers become inert and stimulate a foreign body tissue reaction are not understood. Various conjectures have been offered. Davidsohn<sup>7</sup> considered chronic passive hyperemia of the lungs as a possible etiologic factor, but discarded the idea when he found no fragmentation or calcium deposits in tissues from lungs with changes characteristic of chronic passive hyperemia. He was inclined to ascribe the deposition of calcium to an increase in the calcium in the blood coincident with tumors in bone. However, Kossa<sup>13</sup> demonstrated that when the blood is saturated with calcium there is no deposit in the tissues of the body. In contrast, Katase<sup>14</sup> injected calcium into guinea-pigs and rabbits and noted calcification of elastic tissue in the alveolar septums of the lung. Learner<sup>15</sup> obtained similar results in dogs with injections of parathormone. Davidsohn<sup>7</sup> thought inhalation of dust over long periods of time and motility of the lung until the time of death might be important factors in causing the fragmentation of elastic fibers.

Bittrolff<sup>8</sup> attributed the inflammatory reactions to chronic passive hyperemia. He held that the slow circulation and poor aeration of the blood caused acidosis. The tissues of the lung, particularly the elastic fibers, were thus weakened, and the dyspnea and hyperdistention of the lungs accompanying the venous stasis caused fragmentation of the tissues. As a secondary change iron and calcium were deposited on the degenerated fibers. Giant cells then aggregated about the inert bodies, and deposits of iron and calcium on these fibers formed the concentrically laminated inclusions. Schum<sup>9</sup> did not believe that iron and calcium could be deposited in normal elastic tissue. He thought that an injury to these tissues must have preceded the incrustations. He considered mechanical factors incident to chronic bronchitis and asthma important. He compared the changes in the elastic tissue of the lung to those noted in hypertrophy of the myocardium followed by dilatation. When the elastic limit was reached the tissues ruptured in many places, and as part of the retrogressive changes mineral substances were deposited. He believed that this was an adequate explanation of the changes seen in and about the bronchi, but that chronic passive hyperemia as a sequence of chronic bronchitis was responsible for the similar alterations of the walls of the blood vessels. From his studies Gigon<sup>10</sup> concluded that chronic passive hyperemia in itself was not sufficient to cause the formation of the concentrically laminated bodies incrustated with calcium and iron. Dugge<sup>11</sup> contended that the origin of the giant cells depended on lipoids liberated by the necrotic

13. Kossa, V.: *Beitr. z. path. Anat. u. z. allg. Path.* **29**:163, 1901.

14. Katase, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **57**:516, 1914.

15. Learner, A.: *J. Lab. & Clin. Med.* **14**:921, 1929.

cells of chronic inflammatory tissue. The poorly soluble lipoids thus formed acted as foreign bodies and were phagocytosed after the cholesterol had been changed to mixtures of cholesterol and fatty acid. Calcification of the lipoids he believed to be simply an expression of the affinity of fatty acids for calcium. The deposits of lime on elastic fibers in giant cells he ascribed to the presence of crystals of cholesterol about the fibers.

#### COMMENT

Several possible etiologic factors should be considered in attempting to establish a cause for the lesions in the lungs described here. Among these are marked chronic emphysema, passive hyperemia and edema of the lungs; infection (bronchopneumonia, multiple abscesses and bronchitis); diabetes mellitus, and perhaps the inhalation of a foreign substance, such as oil, although there is no record of this in the clinical history. It is not possible to say with certainty which of these or of their combinations caused portions of the elastic tissue of the lung to become inert, thereby stimulating a so-called foreign body tissue reaction. Chronic passive hyperemia itself does not seem to have been such an important etiologic factor in the production of these lesions, because such changes are not found regularly in the lungs of patients dying with cardiac decompensation. Further, elastic fiber inclusions of giant cells have been found in many places other than the lungs and where venous stasis was not present.<sup>5</sup> The distention of the lungs and the rupture of the elastic fibers with emphysema may be important. There are no statements on record concerning changes in the elastic tissue in diabetes mellitus, but possibly in diabetic lipemia, fats are deposited in tissues and alter them so that they become inert or that the fibers are altered in some way by the acidosis of diabetes. The predominance of the lesions in and about the bronchial tissues suggests the possibility that some foreign (oil) substance may have entered the bronchioles and caused the necrosis which initiated the chemical changes of some of the elastic tissue of the lung. Superimposed infection or infection alone in a diabetic patient may be important.

#### SUMMARY

Granulation tissue containing many multinucleated foreign body giant cells has been found in the lung of a diabetic patient with chronic bronchitis. The inclusions of the giant cells were connective tissue fibers reacting in a modified way with the stains for elastin, but not with the microchemical tests for iron and calcium. Some of these fibers were distributed loosely in the lung without chronic tissue reactions. Emphysema of the lungs and chronic bronchitis seem to have had an etiologic relation to these lesions.

# THE HISTOGENESIS OF AMYLOID BODIES IN THE CENTRAL NERVOUS SYSTEM\*

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## THE RÔLE OF THE MICROGLIA IN THE FORMATION OF THE AMYLOID BODY

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Although numerous investigators<sup>1</sup> have tried to solve the problem of the origin of the amyloid bodies, no definite conclusion has been reached as to the elements that participate in the formation of these characteristic structures.

Here and there mention is found of the origin of amyloid bodies from nerve cells, nerve fibers, glial cells or hematogenous elements. Without entering into the details of the various theories, I shall briefly summarize the views that have been expressed as to their origin.

### THEORIES AS TO ORIGIN OF AMYLOID BODIES

*Neurogenic Theory.*—Among the advocates of a neurogenic origin are Catola and Achucarro,<sup>1</sup> Wolf,<sup>2</sup> Marchand,<sup>3</sup> Scheffer<sup>4</sup> and Stroebe.<sup>5</sup> who expressed the belief that amyloid bodies originate from axis cylinders or from myelin sheaths or from both. Omorokow<sup>1</sup> said that nerve cells must be considered as the source of origin of such bodies.

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1. Extensive bibliographies may be found in the works of Catola and Achucarro (Virchows Arch. f. path. Anat. **184**:454, 1906), Omorokow (Ztschr. f. d. ges. Neurol. u. Psychiat. **100**:109, 1925), Stürmer (Nissl and Alzheimer: Histologische und histopathologische Arbeiten über die Grosshirnrinde, Jena, Gustav Fischer, 1913, vol. 5, no. 3, p. 417), and Siegert (Virchows Arch. f. path. Anat. **129**:513, 1892).

2. Wolf, P.: Die Amyloidkörperchen des Nervensystems, München Dissert., Vienna, Franz Deuticke, 1901.

3. Marchand, F.: Beitr. z. path. Anat. u. z. allg. Path. **45**:161, 1909.

4. Scheffer, quoted by Catola and Achucarro (footnote 1).

5. Stroebe, quoted by Omorokow (footnote 1).

Gamna<sup>6</sup> said that degenerated products originating from axis cylinders and nerve cells may precipitate in the interstitial tissue and form amyloid bodies.

*Gliogenic Theory.*—Some of the investigators, among whom are Redlich,<sup>7</sup> Obersteiner,<sup>8</sup> Nambu,<sup>9</sup> Omorokow<sup>1</sup> and Klebe,<sup>4</sup> have pointed out the probability of the amyloid bodies being developed from the glial elements. They have observed that amyloid bodies are found mostly in locations where the glia cells are most prominent, namely, the subpial and subependymal layers and in the vicinity of the blood vessels.

Nambu, working with Weigert's glia stain, was even able to demonstrate changes in the nuclei of the glial elements which he interpreted as a beginning amyloid change.

Obersteiner pointed to the presence in the glia cells of peculiar globular bodies, which he thought were amyloid material.

In 1927, Uyematsu,<sup>10</sup> in confirmation of a view advanced by Shimoda<sup>11</sup> in 1918, claimed that amyloid bodies are formed through swelling of the prolongments of the astrocytes.

While the majority of authors concerned in the gliogenic theory refer to glia cells in a general way as the source of origin of amyloid bodies, a more definite distinction is found in the work of Ford-Robertson,<sup>12</sup> who first described the so-called mesoglia. In Ford-Robertson's conception, the mesoglia is a glia of mesodermic nature that includes what later was described by del Rio Hortega as the microglia and the oligodendroglia. Among the pathologic conditions that may affect the mesoglia, Ford-Robertson mentioned merely its transformation into an amyloid body. He did not illustrate this transformation, however, with either description or pictures.

*Lymphogenic Theory.*—Among the sustainers of the lymphogenic theory have been Alzheimer,<sup>13</sup> Stürmer,<sup>1</sup> Siegert<sup>1</sup> and Bertrand,<sup>14</sup> who believed that amyloid bodies are a result of the precipitation of the circulating tissue fluids.

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6. Gamna: Arch. per le sc. med. **44**:1, 1921.

7. Redlich: Jahrb. f. Psychiat., 1891, pt. 10.

8. Obersteiner: Arb. a. d. neurol. Inst. a. d. Wien. Univ. 1900, no. 7, p. 301; Anleitung beim Studium des Baues der nervösen Zentralorgane in gesundem und krankem Zustand, Vienna, Franz Deuticke, 1901, p. 237.

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*Hematogenic Theory.*—A few authors think that amyloid bodies are the result of disintegrated blood elements (von Recklinghausen,<sup>5</sup> Touton<sup>6</sup>).

*Postmortem Theory.*—Stilling<sup>5</sup> expressed the belief that amyloid bodies are the result of postmortem changes.

#### OBSERVATIONS

In the course of a pathologic survey of Ammon's horn in epileptic persons I have noticed that in the majority of the cases this structure

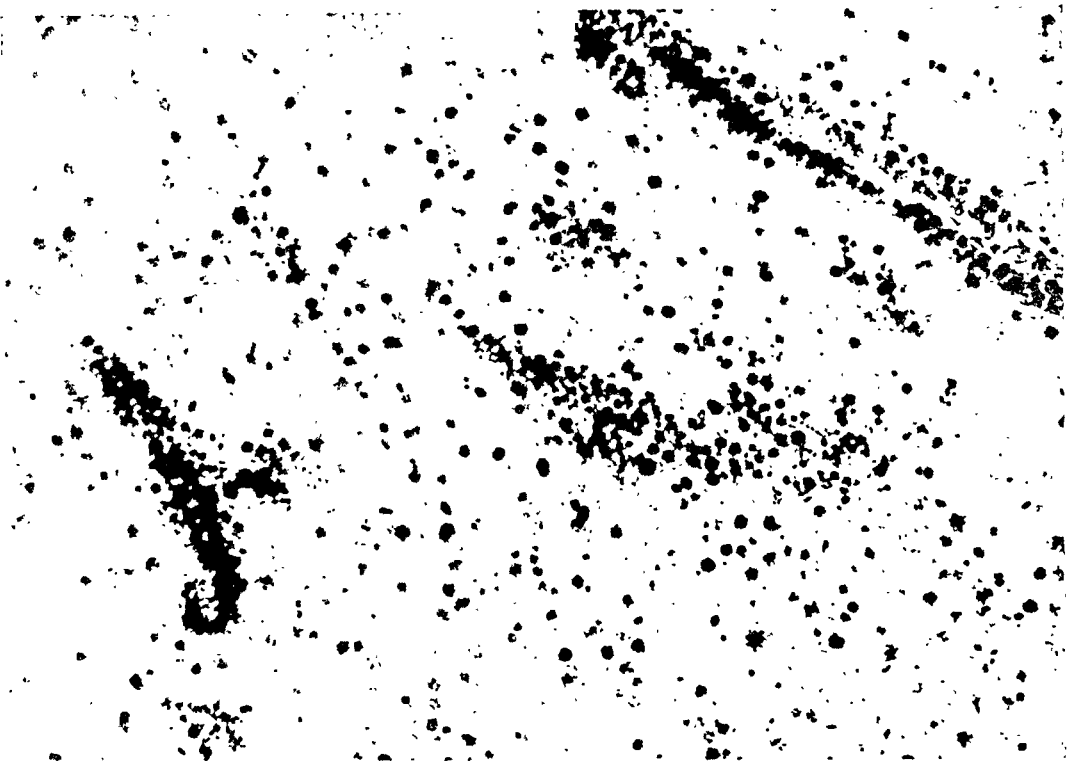


Fig. 1.—Accumulation of amyloid bodies treated with Lugol's solution and sulphuric acid.

presents a considerable number of amyloid bodies. These were definitely proved to be amyloid bodies by their typical staining reaction with iodine and sulphuric acid and also by their reaction to methyl violet. They stain a dark brown with the former, blue with the sulphuric acid, and purplish red with the methyl violet. They also stain blue with the Nile blue sulphate and a pale blue with hematoxylin. With Best's method for glycogen they stain pale red and are not soluble in water.

By del Rio Hortega's method the corpora amylacea are stained different densities of purple, some being very light and others dark, almost black. They are best brought out when the sections are allowed to remain in the gold toning solution somewhat longer than is required in the toning of the glial cells after silver impregnation.

I have been fortunate enough to secure in my material a successful impregnation of the amyloid bodies, which has allowed me to make a thorough study of their morphology and has enabled me to trace the various steps from their origin to their full development.

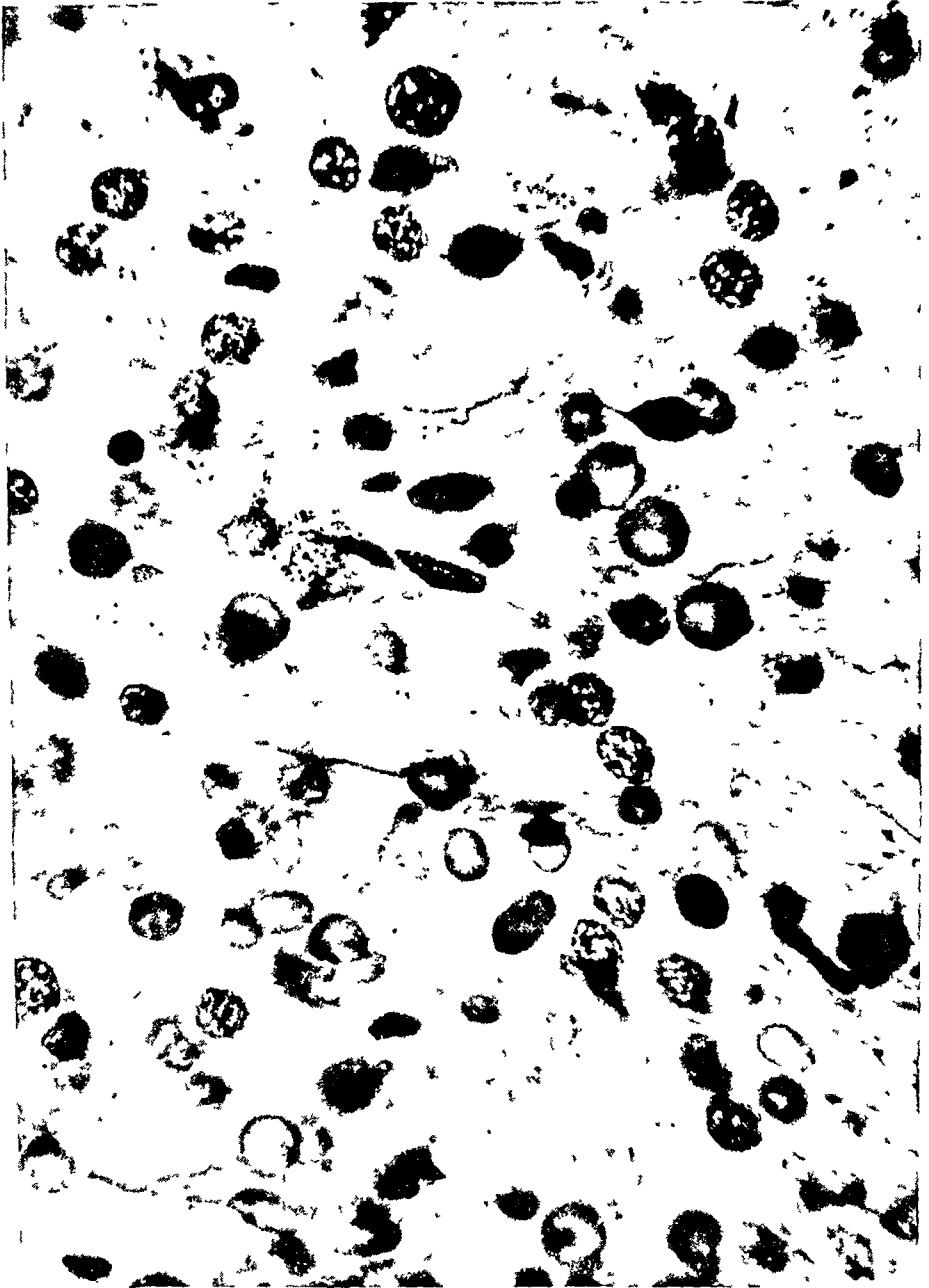


Fig 2—Amyloid bodies This and subsequent figures represent sections impregnated with silver carbonate by the method of del Rio Hortega.

If one looks at a section stained with compound solution of iodine (fig. 1), one is struck by the large number of these amyloid bodies, which are scattered throughout the section, but with a tendency to group themselves along the periphery of the blood vessels.

The area in which the amyloid bodies are found in larger number is the dorsal margin of the fissura hippocampi, an area in which glial cells are normally prevalent, and in the white substance underneath the fascia dentata. In eight of ten epileptic brains that I studied the fissura hippocampi seemed to be the site of predilection for the accumulation of amyloid bodies.

In order to study more carefully the morphology, as well as the derivation of these bodies, I have made extensive use of impregnation with silver by del Rio Hortega's method as modified by Globus and Penfield.

In figure 2, taken from a section impregnated with silver, it may be seen that amyloid bodies are scattered throughout the section and that some of them definitely have processes. The impregnation with silver is more or less intense; some of the corpora amylacea are partly impregnated, while others are almost black. The fact that some of the amyloid bodies disclosed prolongments led me immediately to a more careful study of the cellular elements from which the amyloid bodies might derive.

From a careful study of my slides I reached the conclusion that amyloid bodies are derived from the amyloid degeneration of microglial elements.

The first step in the transformation of a microglia into an amyloid body is a degenerative process of the nucleus. The normal nucleus is usually filled with chromatin bodies located all around the periphery, as well as in the center of the structure (fig. 3). Whenever the amyloid degeneration takes place, the nucleus loses its chromatin mainly in its central portion. Chromatin bodies may still be seen at the periphery of the nucleus, but its central portion acquires a characteristic glassy, homogeneous appearance. Figure 4 illustrates this very early stage of the transformation.

While the nucleus undergoes the amyloid degeneration, changes occur in the cell body and its prolongments. There are two types of reaction of the cell body. The most common one is a breaking down of the cell membrane and the less frequent one is an acute swelling of the cytoplasm.

The prolongments in both cases usually undergo, very early, clasmotodendrosis, that is, fragmentation. Figure 5 demonstrates the fragmentation of the prolongments, whereas, the nucleus of the microglia cell undergoes a more complete amyloid degeneration.

A further step in the production of the amyloid body is the liberation of the degenerated nucleus into the surrounding tissue. This liberation may be followed in its various stages, and formations are seen in which one or more prolongments are still faintly attached to the



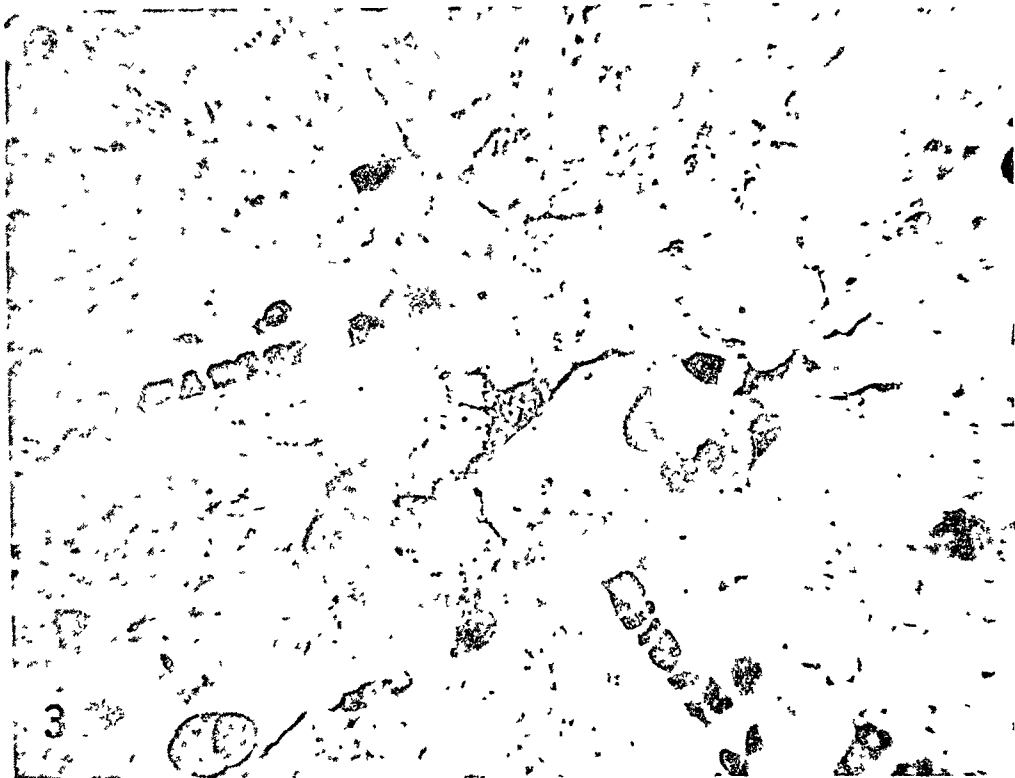


Fig. 3.—Microglial cell the nucleus of which shows normal chromatin content.

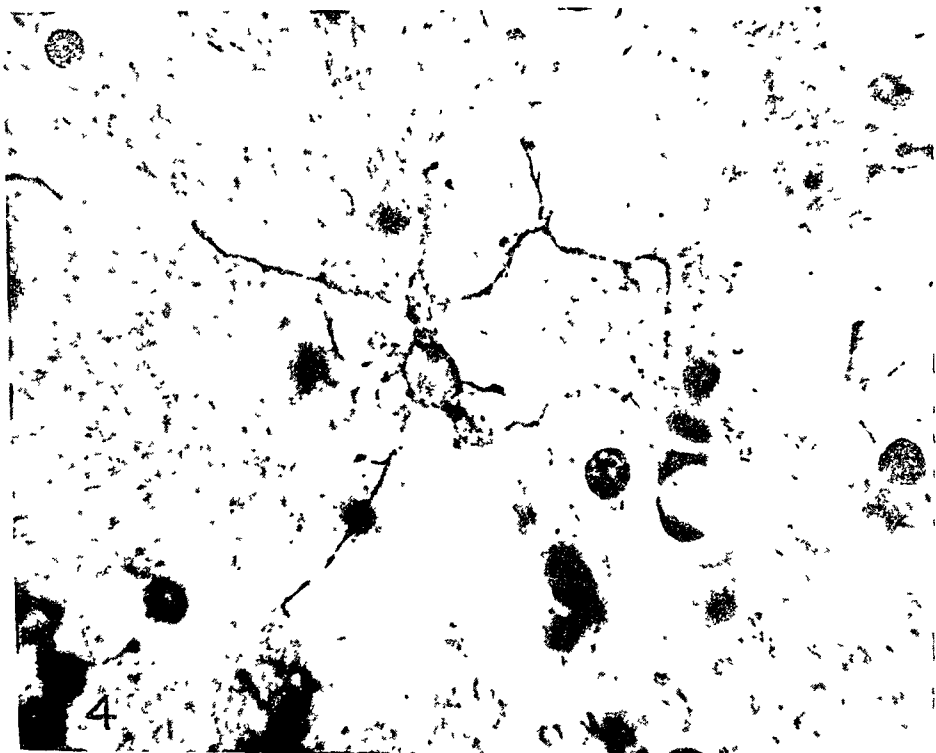


Fig. 4.—The initial stage of the transformation of a microglial cell into an amyloid body. Note the homogeneity and glassy appearance of the nucleus.

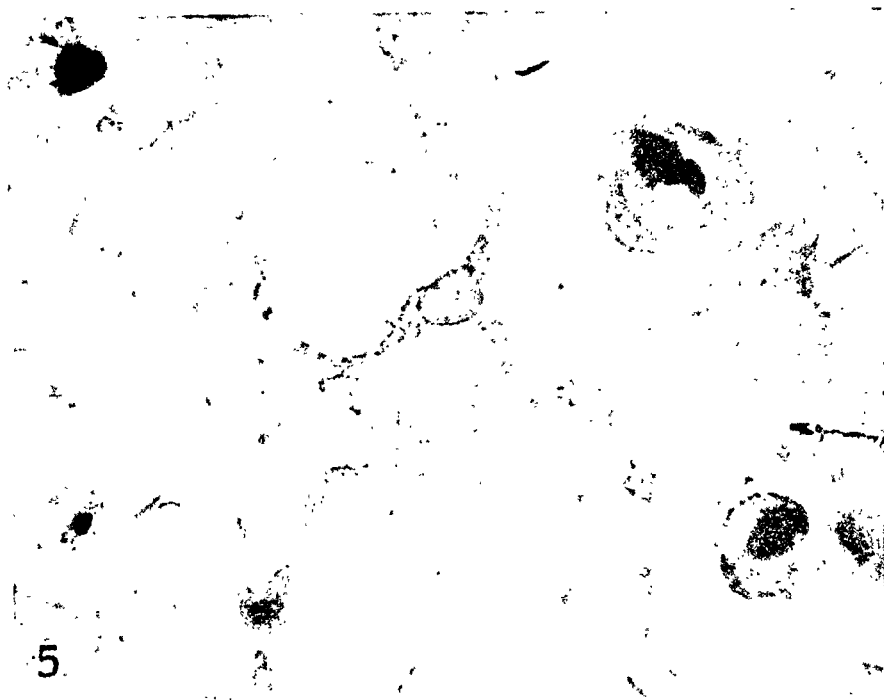


Fig. 5.—A further stage in the transformation of a microglial cell into an amyloid body. Note the appearance of the nucleus and the fragmentation of the prolongments.

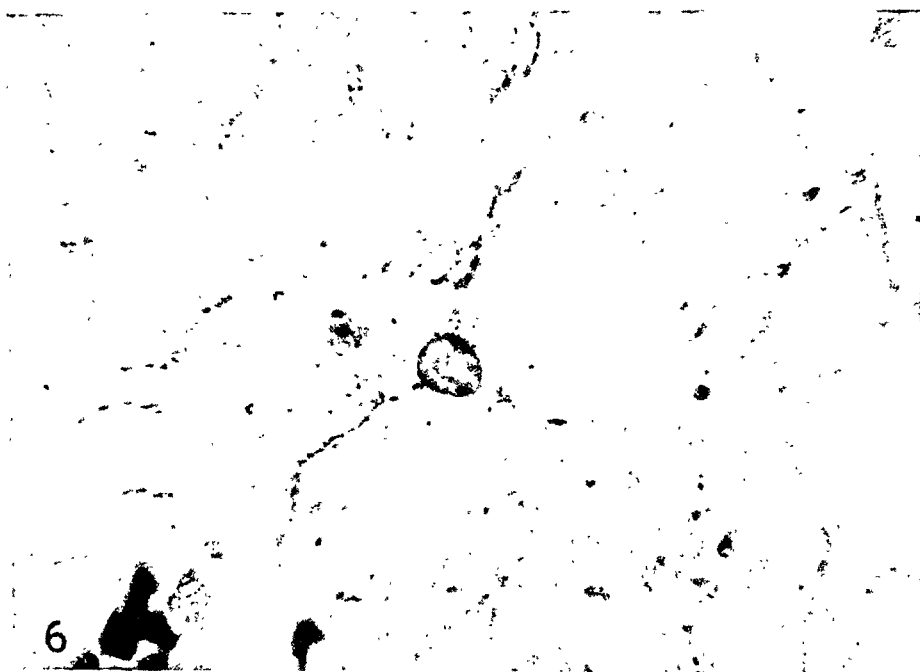


Fig. 6.—A well advanced stage in the transformation of a microglial cell into an amyloid body. Note the almost complete liberation of the nucleus and the faintly attached prolongments.

amyloid body (fig. 6). Rarely it happens that the prolongments also undergo a kind of degeneration, the result of which is the formation of small fragments, some of which are roundish; this degeneration may account for the existence here and there of very small amyloid granules.

In its final transformation into an amyloid body the nucleus of the microglia cell may retain its triangular or oval shape, pointing still to the microglial nature of the transformed substance (fig. 7). Generally, however, the amyloid substance has a tendency to acquire finally a roundish appearance that at a certain point makes its differentiation from the analogous structure derived from the oligodendroglia almost impossible. It is only through the study of the various stages of transformation that one acquires certainty as to the elements from which the amyloid body originates. In some instances, however, the amyloid body still retains an elongated oval shape, the morphology of which recalls the nucleus of the so-called rod cells (*Stäbchenzellen*). It must be said, however, that sometimes this elongated appearance is misleading and derives from the conglomeration and fusion of two or three nuclei, either of microglia or of oligodendroglia cells. As a matter of fact, it is possible here and there still to detect two or three original nuclei in their process of fusion to form an elongated amyloid body (fig. 8).

The other way of transformation of a microglia cell into an amyloid body is the one that produces first a condition of acute swelling of the cellular element.

As already proved by previous work (Meduna,<sup>15</sup> Ferraro and Morrison,<sup>16</sup> Vizioli<sup>17</sup> and others), microglia elements undergo also a degenerative process of the swelling type in which a more or less considerable ring of swollen cytoplasm is found surrounding the nucleus. In the event that the prolongments characteristic of the microglia have already undergone clasmotodendrosis in the stage of roundish transformation of the nucleus, the differentiation between microglia and oligodendroglia becomes at times very difficult. From the stage of acute swelling the microglial cell may gradually undergo amyloid degeneration. The process also originates in the nucleus and leads gradually to the liberation of the amyloid body into the surrounding tissue.

My conception of the transformation of the microglia cell into an amyloid body contrasts with that of del Rio Hortega,<sup>18</sup> who expressed the belief that the amyloid body penetrates into the microglia cells, but is not the result of the degeneration of the cell itself. According to del Rio Hortega the penetration of the amyloid material into the

15. Meduna: *Arch. f. Psychiat.* **82**:123, 1928.

16. Ferraro, A., and Morrison, L. R.: *Psychiatric Quart.* **2**:506, 1928.

17. Vizioli, F.: *Riv. di neurol.* **2**:365, 1929.

18. del Rio Hortega: *Bol. de la Soc. españ. de hist. nat.* **25**:127, 1925.



Fig. 7.—Fully developed amyloid bodies. One of them has a beanlike appearance, recalling the nuclei of some microglial cells.

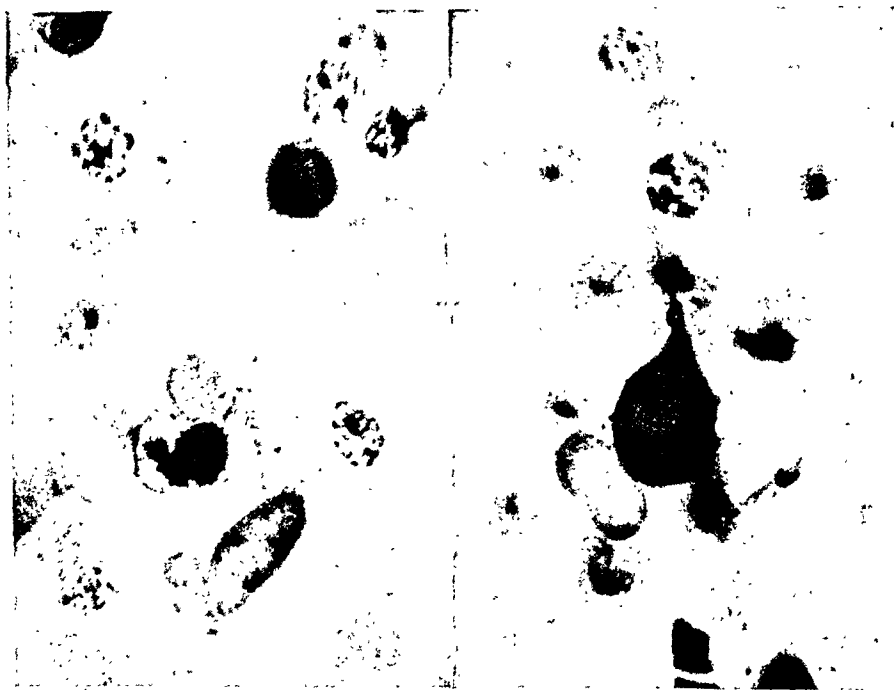


Fig. 8.—Elongated amyloid bodies resulting from the fusion of two or three nuclei.

microglia is not, on the other hand, the result of phagocytosis, but is the result of an impregnation of the cytoplasm, or of a prolongment, of the microglia by amyloid substance, which acquires a roundish aspect only after its condensation inside the microglial element.

According to del Rio Hortega the phases of the penetration of the amyloid into the microglia cell are: (1) occurrence in the interstitial tissue of amyloid material derived from normal metabolism or from disintegrated nerve cells; (2) stasis in the circulation of the interstitial plasma, thus favoring the stagnation of the dissolved amyloid material; (3) collection of the amyloid substance outside the microglial elements; (4) lowering of the cellular tension, thus favoring the penetration of the amyloid substance into the cell plasma; (5) intracellular crystallization of the amyloid body into the microglial cell.<sup>19</sup>

#### THE RÔLE OF THE OLIGODENDROGLIA IN THE FORMATION OF THE AMYLOID BODY

ARMANDO FERRARO AND L. A. DAMON

As pointed out in the previous section, the amyloid body originates from the microglia cell the nucleus of which undergoes a gradual amyloid degeneration. Because of the fact that the oligodendroglia and the microglia both form the so-called third element of Cajal, constituting also the mesoglia of Ford-Robertson, we have centered our attention on the oligodendroglial element, which has not been considered in the first note.

A careful study of numerous sections specifically impregnated with silver carbonate by the method of del Rio Hortega has allowed us to make a fortunate survey of the various stages of transformation of oligodendroglia cells into amyloid bodies.

It seems to us that the first step toward a transformation of the oligodendroglia cell into an amyloid body is generally the condition known as acute swelling, first described by Penfield and Cone. Figure 9 illustrates such an early stage of transformation of the oligodendroglial

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19. A note appearing in the November issue of the Proceedings of the Society for Experimental Biology and Medicine (**28**:172, 1930) reports the results of Grayzel, Jacobi, Maslow and Warshall in experimental amyloidosis. These authors have succeeded in producing diffuse amyloidosis by repeated subcutaneous or intramuscular injections of 5 per cent aqueous suspension of sodium caseinate. In the summary of their results it is reported that amyloid bodies first appeared in the spleen, then in the liver and later in the kidneys. In each instance amyloid substance was first noted within cells of the reticulo-endothelial system. The observations clearly demonstrate the intracellular origin of amyloid substance, thus harmonizing with my own observations of the intracellular origin of the amyloid body in the central nervous system.

cell into an amyloid body. It may be seen that while the cytoplasm is swollen and distinctly recognizable, the nucleus has lost some of its chromatin bodies and has acquired a particular glassy, homogeneous appearance. The prolongments may still be seen in moderate amount and undergoing fragmentation.

In a further stage the degenerated nucleus invades more or less the whole cellular element, and the amyloid body would acquire its whole individuality were it not for the presence of one or two prolongments that still establish the cellular origin of the structure. Figures 10, 11 and 12 illustrate the occurrence of amyloid bodies to which

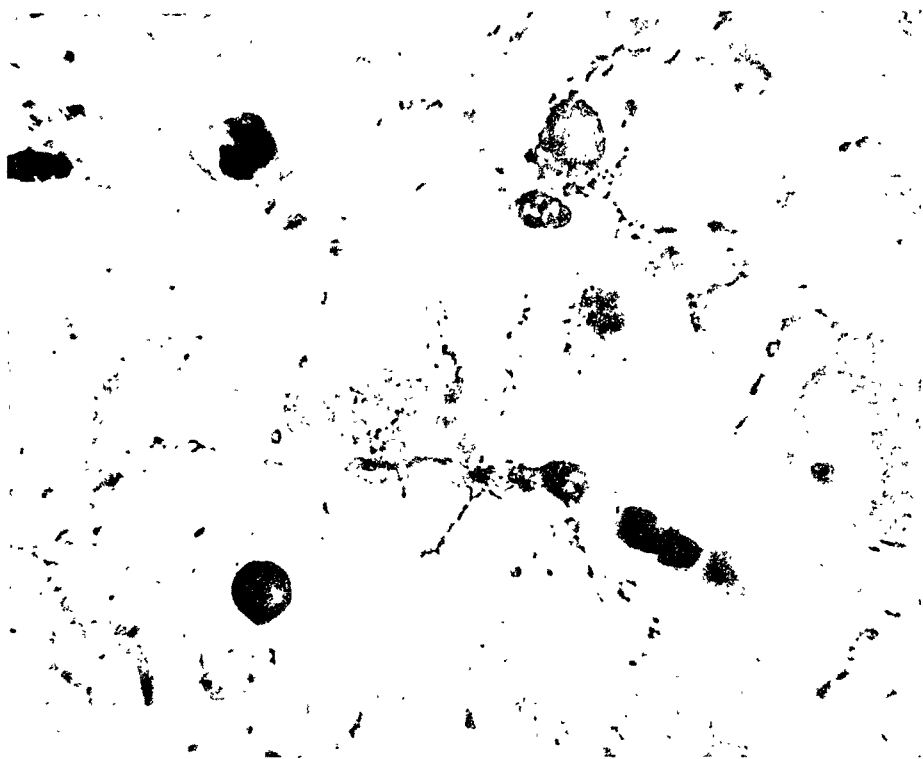


Fig. 9.—Swollen oligodendroglial cells with nuclei undergoing amyloid degeneration.

two, and later, one, prolongment still remains attached. Figure 10 clearly shows that a certain amount of cytoplasm is still visible at the site of origin of one of the two prolongments. The cellular nature of the element from which the central structure derives cannot be questioned in the aforementioned case. In figure 11 a definite amount of swollen cytoplasm is still detectable over the upper portion of the amyloid body opposite to the origin of the process that is still attached to the cell. Figure 12 illustrates also the occurrence of a small vacuolated ring of cytoplasm still surrounding the amyloid body, to which a long prolongment is attached. Morphologically, no difference is

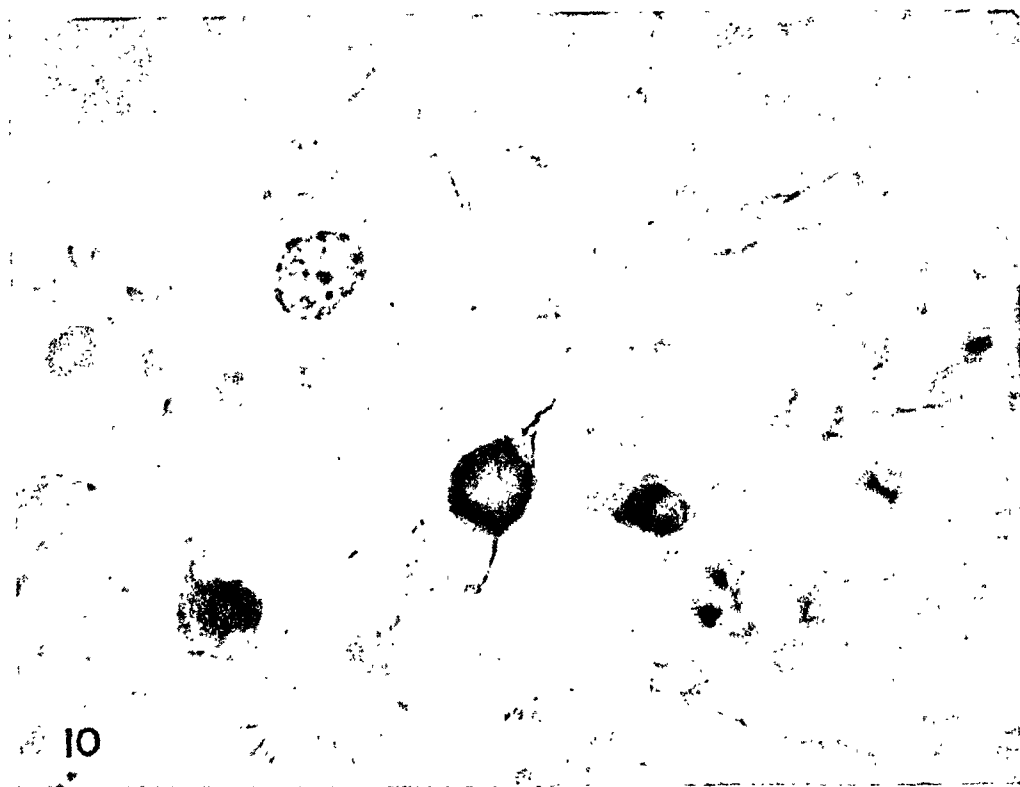


Fig. 10.—Amyloid degeneration of an oligodendroglial cell still possessing two prolongments.

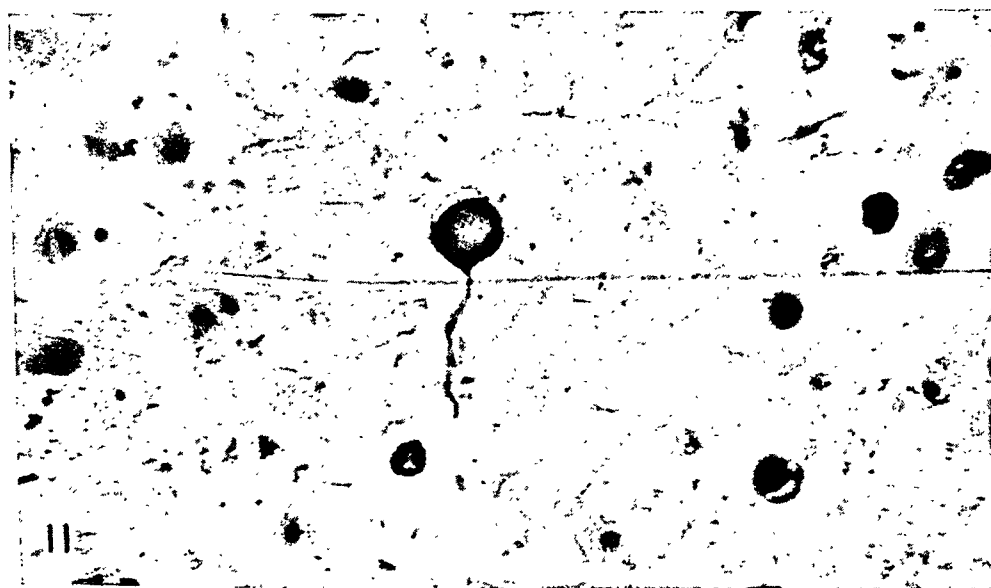


Fig. 11.—Amyloid degeneration of an oligodendroglial cell still showing one prolongment; swelling of part of the cytoplasm is also demonstrated.

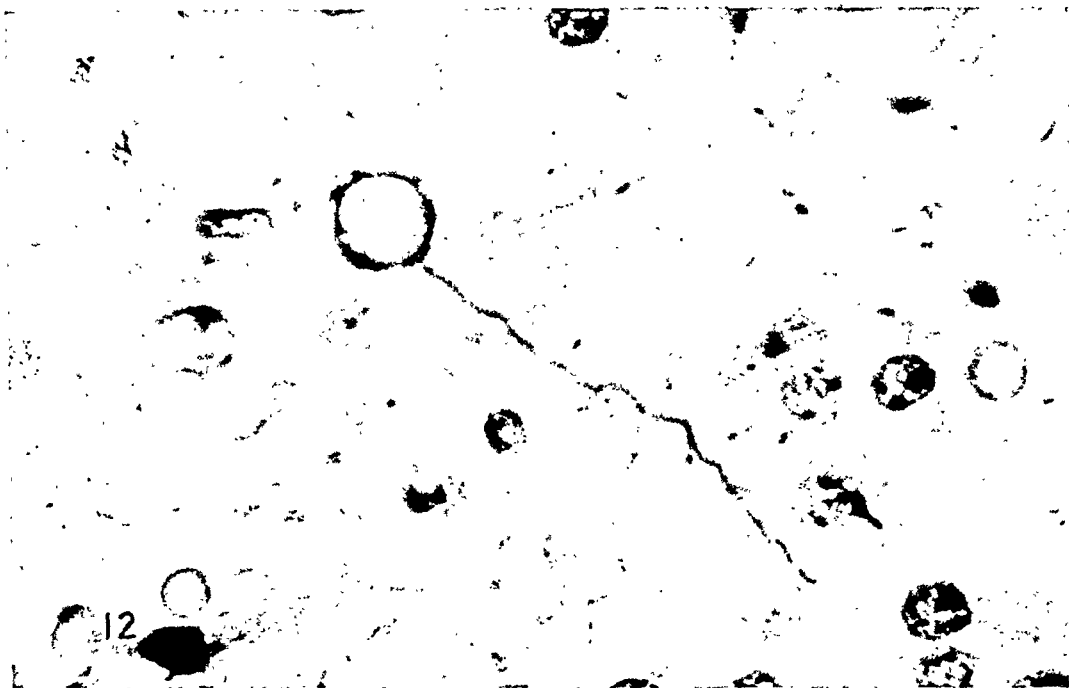


Fig. 12.—Amyloid body with prolongment pointing to its cellular origin.

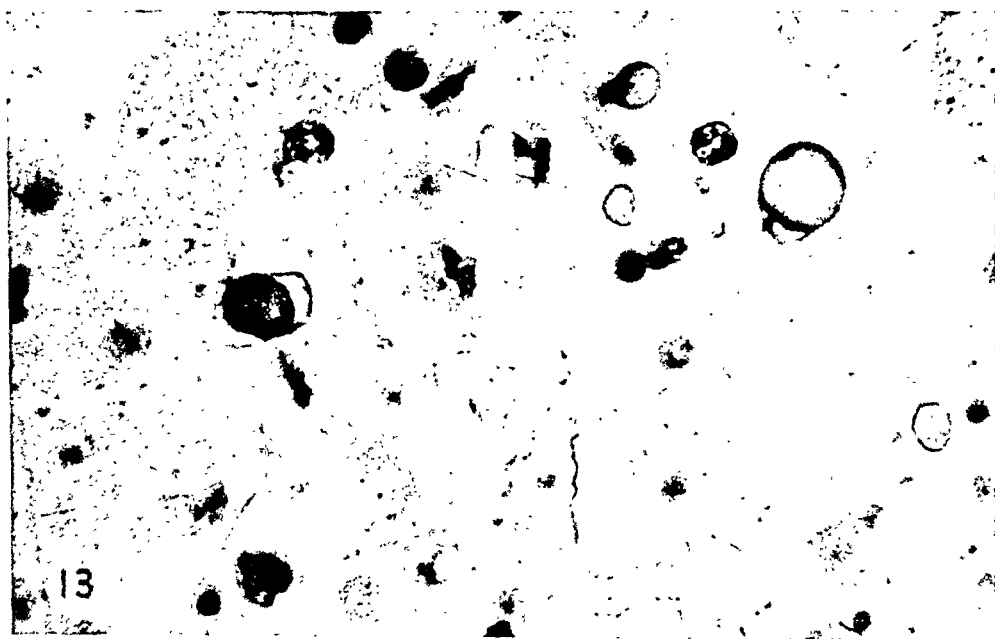


Fig. 13.—Swollen oligodendroglial cell containing a full-formed amyloid body derived from the nucleus of the cell.



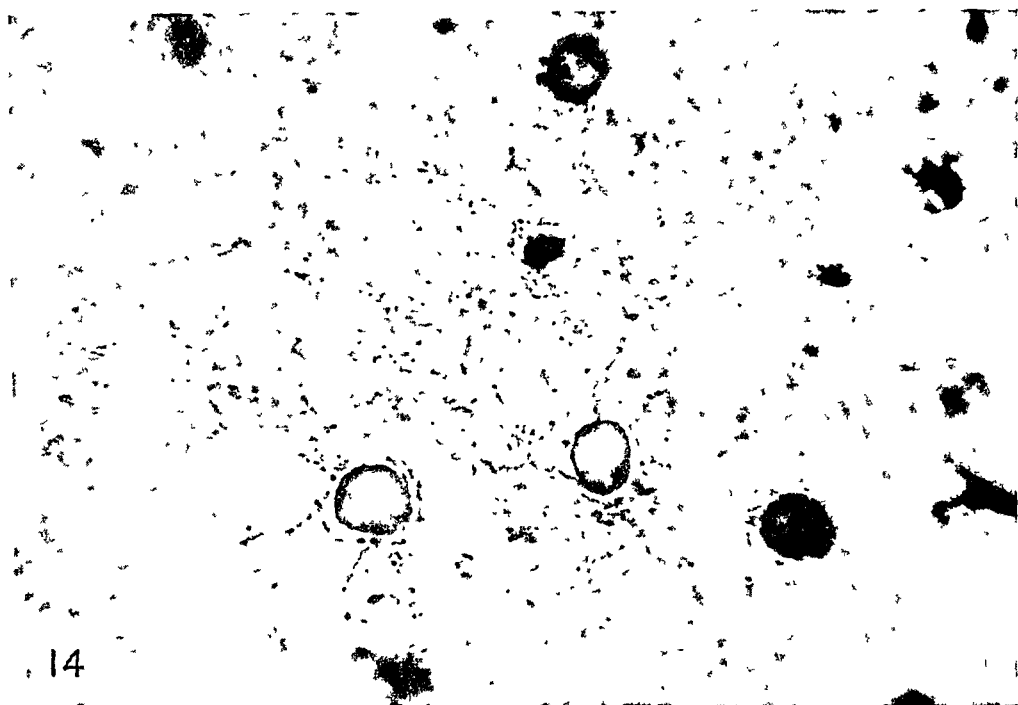


Fig. 14.—Two amyloid bodies contained in the remnants of cellular structures undergoing fragmentation.

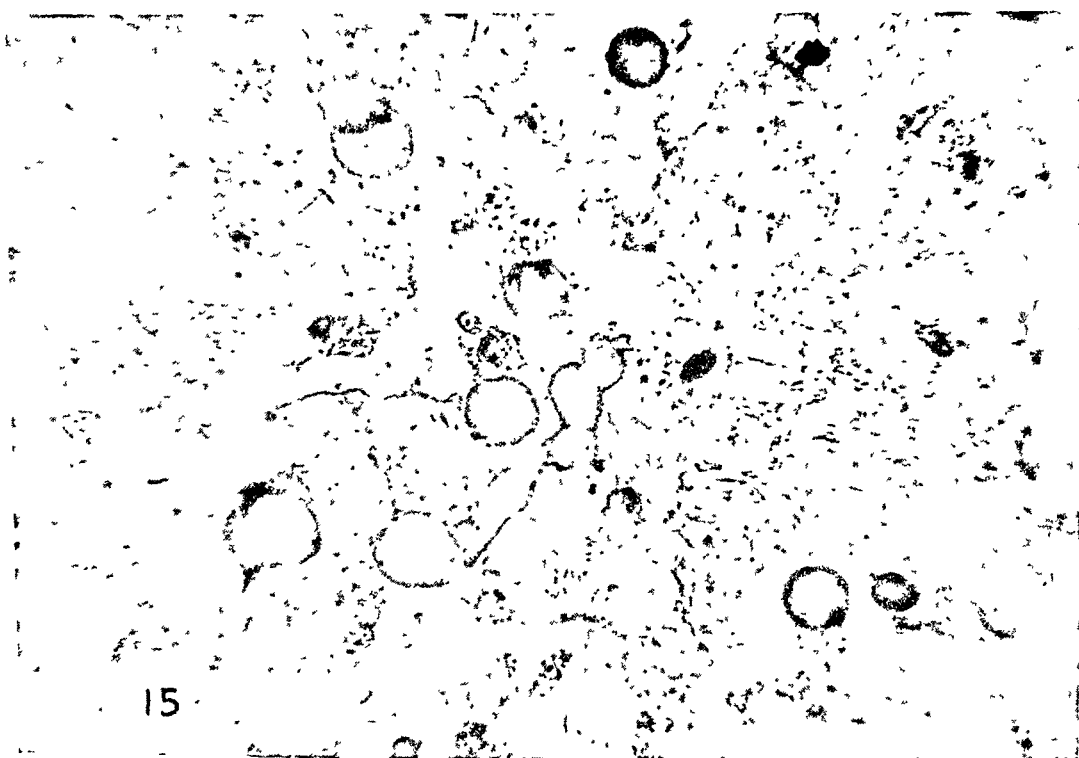


Fig. 15.—Three amyloid bodies collected in a cluster, recalling the normal collection of oligodendroglial cells.

evident between the amyloid body in question and the other smaller ones scattered in the same field.

At a later stage the oligodendroglia cell loses all its prolongments, and the amyloid body may appear located at the center of the cellular formation surrounded by more or less cytoplasm clearly pointing to the nuclear origin of the central mass. The resulting picture is morphologically identical with the acute swelling that occurs in oligodendroglia cells, the difference residing in the chemical reaction of the nucleus.

That the structure from which the amyloid body derives is a cellular structure is also proved by the fact, as shown in figure 14, that the amyloid body may at times be present in the center of a cellular element



Fig. 16.—Collection of normal oligodendroglial cells in clusters of two and three elements.

undergoing a process of breaking down of both cell membrane and prolongments. There is no doubt in our mind as to the cellular nature of the original elements, nor of the amyloid nature of the central bodies, which are identical with other free amyloid bodies detectable in the same section.

We have also centered our attention on finding collections of two or three amyloid bodies in various stages of formation, recalling the normal collection of oligodendroglia cells. Figure 15 clearly demonstrates the collection of three amyloid bodies, one of which still retains a few prolongments, recalling with striking identity the collection of oligodendroglia cells as it occurs in normal tissue (fig. 16).

In conclusion it may be said that by the method of del Rio Hortega one is able to follow the sequence of events in the production of corpora amylacea from the oligodendroglia cells. The advocates of other origins

of the amyloid bodies have failed to demonstrate a like sequence of events. That the amyloid bodies are developed from the nucleus of the oligodendroglia seems to be fairly well brought out by our investigation. Whether the oligodendroglia is the only element in addition to the microglia from which the amyloid bodies can develop is a question that should be left open for further investigation. All that we can say is that, at least in the brain, we have failed to encounter other structures that would suggest a transformation into amyloid bodies. Control staining by the Nissl method, the Cajal method or the Bielschowsky method for axis cylinders has been unsuccessful in our hands in establishing any derivation of the amyloid bodies from such structures. Our aim has been to establish only the new fact that, irrespective of their sources, oligodendroglial cells degenerate into amyloid bodies and that the degeneration is primarily a degeneration of the nucleus. This degeneration can be especially well studied in cases of epilepsy, particularly in Ammon's horn, as this region has already been recognized as one of the most sensitive regions of the brain owing to a defective vascular supply (Uchimura<sup>20</sup>). It is possible that through repeated injury due to paroxysmal vasospasm insufficient nutrition takes place, which is followed by the degeneration of oligodendroglia into amyloid substance. Whether or not the first stage of reaction is always a condition of acute swelling is a matter for speculation, but while acute swelling is a reversible pathologic condition, once the amyloid degeneration has taken place in the cellular element the condition is presumably permanent.

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20. Uchimura, J.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **109**:501, 1927.

# SEPTIC PERITONITIS IN MAN DUE TO BACILLUS SORDELLII

REPORT OF A CASE \*

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AND

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To the present, only seven strains of *Bacillus sordellii* have been reported. The first two of these were recognized in man in 1922 by Sordelli <sup>1</sup> at Buenos Aires in a gangrenous postoperative infection and in a gangrenous wound following a fracture, and in view of the apparently intermediate position of the organism between *B. oedematis* (*B. novyi*) and *B. sporogenes*, he named it *Bacillus oedematis-sporogenes*.<sup>2</sup>

A second series of three cultures was recovered by Meleney, Humphreys and Carp from a fatal postoperative infection and from two samples of catgut in the Presbyterian Hospital in New York. Not knowing of Sordelli's work, and supposing they had discovered an entirely new species of anaerobic bacillus, the New York investigators gave their cultures the name *Clostridium oedematoides*.<sup>3</sup> In the meantime, Hall and Scott <sup>4</sup> had confirmed the distinctive properties of *B. oedematis-sporogenes*, but considering the trinomial to be invalid and both of its components to be easily confused with names of other species, if used in a binomial, they renamed the South American cultures *Bacillus sordellii* in honor of their discoverer, and on purely morphologic and cultural grounds suggested the possibility of an identity with *Cl. oedematoides*. Their actual identity was later proved by careful morphologic and cultural comparisons and by tests for cross-protection with specific antitoxins; this research was carried on independently by Humphreys and Meleney <sup>5</sup> and by Hall, Jungherr and Rymer.<sup>6</sup>

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\* Submitted for publication, Jan. 12, 1931.

\* From the departments of bacteriology and public health, and pathology, University of Colorado School of Medicine and Hospitals.

1. Sordelli, A.: Rev. Asoc. méd. argent. **35**:308, 1922; Compt. rend. Soc. de biol. **87**:838, 1922; **89**:53, 1923.

2. Sordelli, A.: Rev. Asoc. méd. argent. **36**:52, 1923; Rev. del Inst. Dept. Nac. de hig. **3**:1, 1923.

3. Meleney, F. L.; Humphreys, F. B., and Carp, L.: Proc. Soc. Exper. Biol. & Med. **24**:675, 1927; Surg. Gynec. Obst. **45**:775, 1927.

4. Hall, I. C., and Scott, J. P.: J. Infect. Dis. **41**:329, 1927.

5. Humphreys, F. B., and Meleney, F. L.: Proc. Soc. Exper. Biol. & Med. **25**:611, 1928.

6. Hall, I. C.; Jungherr, E., and Rymer, M. R.: J. Infect. Dis. **45**:42, 1929.

Hall<sup>7</sup> later received two additional cultures from L. R. Vawter of Reno, Nev., which had been isolated in 1921 from cattle suffering with icterohemoglobinuria; these were shown to be morphologically, culturally and serologically similar to the South American and New York strains.

We now report a new strain recovered from the heart blood and peritoneal cavity at autopsy in a case of peritonitis in which no operation was performed. We thus record the probability of the occasional origin and residence of *B. sordellii* in the intestinal tract of man, as well as its occurrence in a fourth geographic location. This is also believed to be the first instance in which there has been described an infection with *B. sordellii* without previous operative or recent external trauma.

#### ABSTRACT OF CASE HISTORY

J. C., an obese Negro, aged 55, admitted to the hospital on Sept. 17, 1929, gave a history of syphilis and gonorrhea and of a paralytic stroke in April, 1928, with partial recovery followed by periods of intermittent delirium. The results of the physical and routine laboratory examinations have no particular significance in relation to the terminal infection and are therefore omitted. Suffice it to say that a diagnosis of chronic myocarditis with decompensation, hypertension and arteriosclerosis was made.

The patient was kept in bed, but with difficulty, owing to his mental confusion. He was placed on a milk diet and given epsom salts and digitalis and repeated colonic irrigations. There was considerable incontinence, and the odor of the feces was offensive.

During the first two weeks the temperature fluctuated between 98 and 100 F.; his general condition gradually grew worse, and he died on October 6. Forty-eight hours before death the temperature, which had been normal, began to rise, reaching 101 F. in about twenty hours; it then dropped back to normal, but quickly shot up to from 104 to 104.4 F., at which it remained until shortly before death. During the last four days the pulse rate fluctuated between 110 and 130. The respiratory rate also increased from a previous average of about 22 to about 40 a minute twenty-four hours before death, but the breathing was so shallow toward the end that the respirations could scarcely be counted. Simultaneously with the rise in temperature much mucus and later bright blood appeared in the stools and continued to ooze from the rectum until the patient died.

*Autopsy.*—The body was cooled as quickly as possible to about 4 C.; autopsy was performed twenty-two and one-half hours after death. Only those features of the gross and microscopic picture that might have a bearing on the bacteriologic observations will be mentioned.

The peritoneal cavity contained about 1,000 cc. of slightly yellow, turbid fluid. Two sections of the sigmoid were markedly reddened, and almost hemorrhagic, and one of these was adherent to the iliac crest by fresh fibrinous exudate. The peritoneum was also slightly injected. There were a few old pleural adhesions posteriorly, but no free fluid. There was a small amount of straw-colored fluid in the pericardium.

7. Hall, I. C.: J. Infect. Dis. **45**:156, 1929.

The diagnosis of chronic heart disease with hypertension was confirmed. There was no macroscopic or microscopic evidence in the heart of the specific terminal infection.

The right lung weighed 610 Gm. The posterior and upper part of the upper lobes were deep red and were somewhat raised; on section a moderate amount of fluid could be expressed. Microscopically, the alveoli were emphysematous, and in places the walls were thickened. Some of them contained serum and a few polymorphonuclear leukocytes. The walls of the blood vessels were thick. The left lung weighed 370 Gm., varied in color from gray to pink, mottled with black, and contained air throughout, with emphysema in many sections.

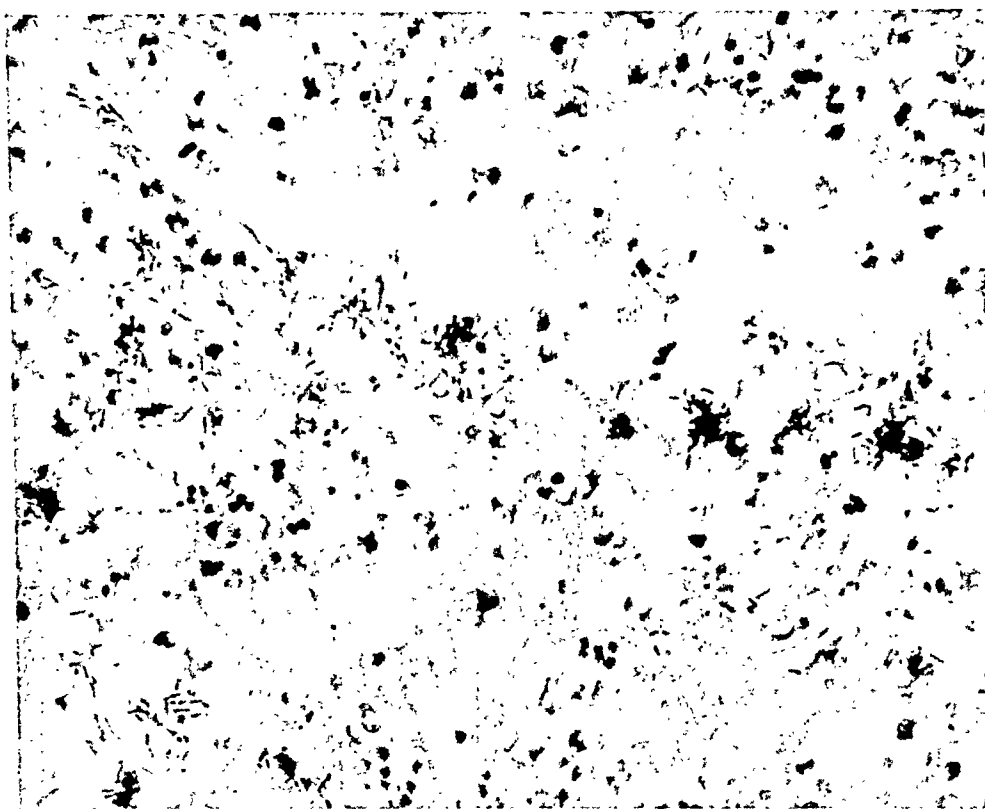


Fig. 1.—Photomicrograph showing gram-positive bacilli, thought to be *B. sordeλλii*, diffusely distributed through the submucosa of the sigmoid, as seen with the high dry lens (Gram stain).

There were a few small hemorrhages in the ileum and jejunum. The sigmoid was adherent to the peritoneum near the crest of the ileum by a fresh fibrinous exudate, and both sigmoid and rectum contained three circumscribed deep red areas with thickened and ulcerated mucosa. On section the entire wall was swollen, hemorrhagic and necrotic, and at one point there had been an acute inflammatory reaction over its peritoneal surface. At this point the entire wall was a thick layer of edematous hemorrhagic tissue, in which one could see dull gray or opaque yellow streaks. This process extended to the peritoneum and at the site of the peritonitis extended through it. The blood vessels in the region of these areas showed no change.

Several sections taken through the intestine, especially at the site of the local peritonitis, revealed an infection of the entire wall.

The mucosa of the sigmoid was entirely necrotic and was replaced by a sero-fibrinopurulent layer with clumps of bacteria deposited on the surface.

The submucosa was thick and edematous and laden with polymorphonuclear leukocytes, zones of hemorrhage and small clumps of bacteria. The upper part of the submucosa was necrotic, but less so than the mucosa.

In the muscular coat only few good muscle cells were seen. For the most part, the cells were vacuolated or had been entirely destroyed by an exudate similar to that in the submucosa, but less intense.

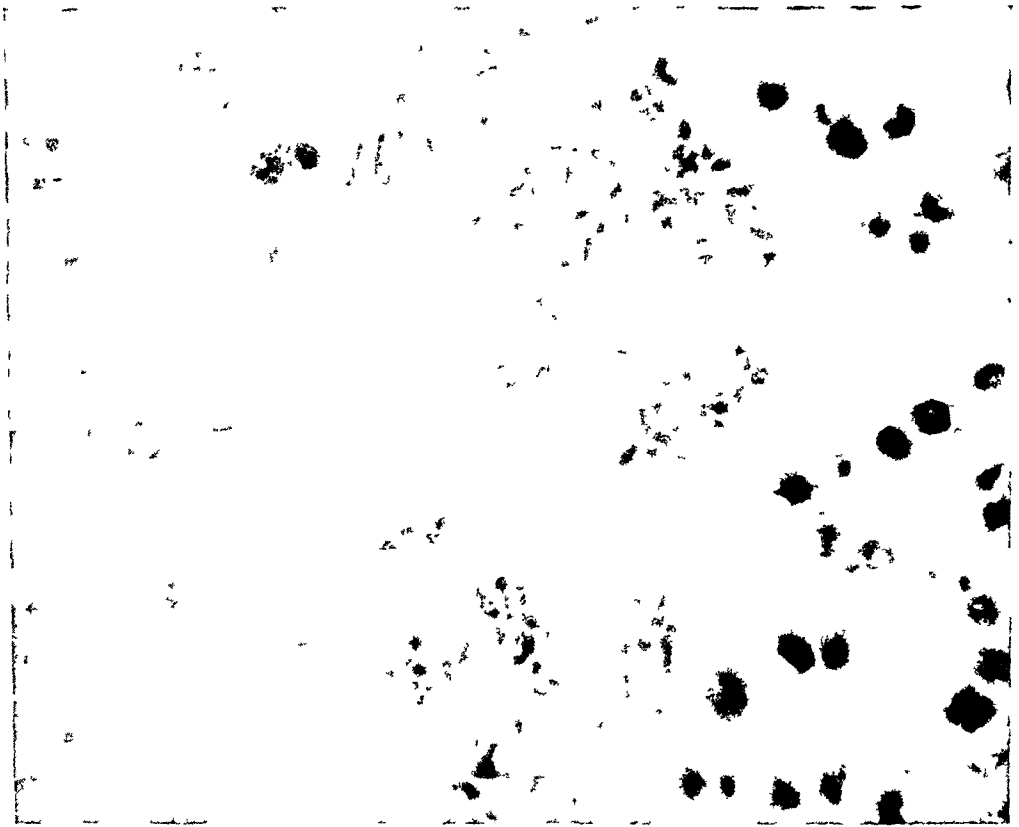


Fig. 2.—Photomicrograph showing gram-positive sporulating bacilli, thought to be *B. sordellii*, in the submucosa of the sigmoid as seen with the oil immersion lens (Gram stain).

The serosa was also the seat of an inflammatory reaction, and the walls of several of the arteries were necrotic and infiltrated by polymorphonuclear leukocytes.

Tissues stained by Gram's method showed large masses of gram-positive bacilli and cocci and gram-negative bacteria in the mucosa, but only large gram-positive bacilli with subterminal spores in the submucosa, muscularis and serosa (figs. 1 and 2).

The liver weighed 1,350 Gm, and was reddish brown and firm, with indistinct markings on section. The central veins were congested, and the adjacent cells had apparently undergone postmortem necrosis and vacuolization.

*Bacteriologic Examination.*—Unfortunately no bacteriologic work was done prior to death. At autopsy blood was secured from the heart by means of a sterile Pasteur pipet plunged through a seared area on the right ventricle. As in most cases of septicemia, the blood was still unclotted. Direct microscopic examination of stained smears showed the presence of numerous large gram-positive rods.

Primary culture was made in deep brain medium; on incubation gas and turbidity developed, which were shown to be due to a mixed culture of thick gram-positive rods and slender gram-positive rods. Subculture on a blood agar plate proved that no aerobic bacteria were present, and the failure of a culture in milk (constricted tube) to show the stormy fermentation characteristic of *B. welchii* enabled us to exclude the presence of this organism. A difference in the size of the colonies in deep agar enabled us to distinguish between the two species of anaerobic bacilli that were present, and by repeated picking of such colonies we secured pure cultures of each.

The slender rod forming minute colonies proved to be motile and to have an oval terminal spore. It fermented dextrose and salicin, with the formation of acid and gas, but not lactose, and failed to produce any black discoloration deeply in a tube containing iron-brain medium; neither did it liquefy gelatin on incubation for one week. A guinea-pig weighing 250 Gm. inoculated with 2 cc. of a seven day dextrose broth culture indicated a lack of pathogenicity. This organism was apparently identical with one that we have not infrequently recovered from peritoneal fluid and occasionally from heart blood in cases of peritonitis, but its actual identity is still undetermined. It is closely related to *B. tetanomorphus*, but differs from the latter in its failure to liquefy gelatin and in its fermentation of salicin. It is considered to have been a secondary invader of no importance.

We were unprepared to find so unusual an organism as *B. sordellii*. The preliminary observation of the pure culture containing the larger rod, in deep agar, on blood agar slants under alkaline pyrogallol, and in milk and gelatin in constricted tubes, suggested either *B. bifermentans* or *B. centrosporogenes*, which we have occasionally recovered as secondary or incidental invaders in peritonitis; but these species are nonpathogenic for laboratory animals, and it was only the marked and characteristic action of the organism on subcutaneous inoculation into guinea-pigs that called our attention to the true identity of this new strain of *B. sordellii*, which was later confirmed by the toxin-antitoxin tests. This experience showed the close relationship of these species and suggested that not too much reliance can be placed on the usually subterminal position of the spore in *B. sordellii* as a means of differentiation from *B. centrosporogenes* and *B. bifermentans*, because young spores of *B. sordellii* are apt to be centrally located, as we<sup>6</sup> have previously pointed out. We emphasize the necessity of tests for virulence and particularly tests for protection with specific antitoxin in the recognition of this species. At the same time, it should be recalled that one of our South American strains of *B. sordellii* has never shown any sign of virulence in our hands, but such strains can be distinguished from *B. centrosporogenes* by the agglutination test.<sup>8</sup>

It suffices here to say that the larger rod from the heart blood showed all of the usual morphologic and cultural properties of *B. sordellii* and was definitely identified by means of a horse serum prepared against our South American strain of *B. sordellii*, and also by means of a rabbit serum prepared against the New York strain.

8. Scott, A. L.: Univ. Colorado Stud. **30**:102, 1930.



# Summary of Experiments on Animals in Case of Septic Peritonitis Due to *Bacillus Sordellii*

Date	Guinea-Pig Weight, Gm., Dose, Cc.	Material Injected *	Antiserum (Dosage 1 Cc.)	Route of Infection	Results	Heart Blood Culture
Oct. 31	200	24 hr. dextrose broth culture 4	.....	Intraperitoneal	Nov. 1, guinea-pig found dead: edema of subcutaneous tissues, pleura and peritoneum; adrenals congested	Sterile
Nov. 2	350	48 hr. dextrose broth culture 3	.....	Subcutaneous	Nov. 3, not seen. Nov. 4, found dead; marked subcutaneous edema; slight congestion; adrenals markedly congested	(No culture made)
Nov. 5	700	4 day dextrose broth culture 3	A serum prepared against <i>B. sordellii</i> 1	Subcutaneous	Nov. 6, very slight edema. Nov. 7, edema subsided; lived	.....
Nov. 5	740	4 day dextrose broth culture 3	.....	Subcutaneous	Nov. 6, marked subcutaneous edema. Nov. 7, found dead; marked subcutaneous edema; adrenals congested	<i>B. sordellii</i>
Nov. 7	250	7 day dextrose broth culture 3	.....	Subcutaneous	Nov. 8, slight subcutaneous edema. Nov. 9, recovered; lived	.....
Nov. 10	340	3 day dextrose broth filtrate 3	.....	Subcutaneous	Nov. 11, marked edema. Nov. 12, found dead (about 11 hr.); marked subcutaneous edema; adrenals congested	Sterile
Nov. 10	400	3 day dextrose broth filtrate 3	Rabbit serum prepared against <i>B. sordellii</i> 2	Subcutaneous	Nov. 11, slight edema. Nov. 12, better. Nov. 13, eyes clouded. Nov. 14, eyes clear; lived	.....
Nov. 10	400	3 day dextrose broth filtrate 4	.....	Subcutaneous	Nov. 11, marked edema early; died late in day: extensive subcutaneous edema; adrenals congested	Sterile
Nov. 10	350	3 day dextrose broth filtrate 4	Rabbit serum prepared against <i>B. sordellii</i> 2	Subcutaneous	Nov. 11, moderate edema. Nov. 12, edema subsided. Nov. 13, eyes clouded. Nov. 14, eyes clear; lived	.....
Nov. 10	275	3 day dextrose broth filtrate 2	.....	Subcutaneous	Nov. 11, marked edema. Nov. 12, found dead (about 11 hr.); marked subcutaneous edema; adrenals congested	Sterile
Nov. 10	250	3 day dextrose broth filtrate 2	Rabbit serum prepared against <i>B. sordellii</i> 2	Subcutaneous	Nov. 11, marked edema. Nov. 12, edema subsiding. Nov. 13, eyes clouded. Nov. 14, eyes clear; lived	.....

\* Sources of cultures used in these experiments: *B. sordellii* 1, South American strain 1303 from malignant edema; *B. sordellii* 2, New York strain 1317 from catgut; *B. sordellii* 3, strain 3572Aa from heart blood in septic peritonitis, reported on in this paper, and *B. sordellii* 4, strain 3572 Ba from peritoneal fluid in septic peritonitis reported on here.

An identical organism was present apparently in pure culture in the peritoneal fluid, which was carefully collected, precautions being taken to avoid its contamination through contact with the pathologist's fingers and knife. This fluid was slightly turbid and slightly green. Microscopically, it contained numerous large gram-positive rods; the primary culture of these appeared to be pure and was likewise identified as *B. sordellii*.

Neither culture differs in any respect from South American, New York and Nevada strains, as described in the literature, and it is therefore unnecessary to repeat the description of their properties in detail, for which we may refer the interested reader to the previous publications. We call attention, however, to the protocol of our tests for virulence and antitoxic protection as summarized in the table.

Inspection of the table shows that in each series of tests the protected animal was always smaller than the unprotected control, but received a larger dose of culture or filtrate, in some cases five times as much. This arrangement of the experiments conclusively demonstrated the protective effect of the antiserums and confirmed the identity of the new cultures.

#### COMMENT

The final cause of death in this patient was apparently an invasion into and through the intestinal wall by *B. sordellii*, resulting in peritonitis and septicemia with the profound toxemia that characterizes the action of this micro-organism. The intestinal lesion that we found was essentially similar to the infected wound described by Meleney, Humphreys and Carp, as indicated by the extensive edema of the intestinal wall, the infiltration by polymorphonuclear leukocytes, the necrosis of the muscularis and the irregular distribution of the bacilli.

Although the liver showed numerous small areas of necrosis, lack of tissue reaction distinguished the changes which we observed in this organ from those observed by Meleney and his associates. We are inclined to regard the changes in the liver in our case as essentially post mortem.

Tissues from all of the organs were stained by Gram's method for bacteria, but bacteria could be detected only in the suprarenal glands, pancreas and kidneys, and it is noteworthy that in these tissues they were in the fat and connective tissue around the organs and extended into them from the outside only at one or two points. No inflammatory reaction was observed in any of these organs on examination of tissues; neither were bacteria demonstrable in the capillaries of the lungs, and their presence in the suprarenal glands, liver, pancreas and kidneys should probably be regarded as due to postmortem multiplication of bacteria present in small numbers in the blood stream at the time of death. Thus the process was essentially a fulminating sigmoiditis and proctitis, with terminal peritonitis, septicemia and toxemia due to invasion of the intestinal wall by *B. sordellii* from the contents of the intestine.

We are not inclined to think that *B. sordellii* will be frequently found as an infecting agent in man. Dr. Hall has been actively engaged for the past eighteen years in collecting and identifying cultures of anaerobic bacilli from various sources, without encountering any strains other than those that we have mentioned. This experience has covered numerous cultures from all parts of the United States and of Europe and from South America, as well as many cultures previously isolated from human sources. Thus the present case is the only one in which this organism was demonstrated in more than 250 autopsies on man and examinations of infected wounds, carefully analyzed for anaerobic bacilli during the past five years. The fact that *B. sordellii* forms highly resistant spores and is easily cultivated in all of the mediums and devices commonly used for anaerobic bacteria suggests that if it were common in nature it would have been detected long ago and would have been found by many investigators instead of by only four groups. Yet its great virulence and the possibility of its occurrence in infected wounds and in obstructive and postoperative peritonitis seem to us to justify the inclusion of its antitoxin in the polyvalent serums that are now being advocated in the prophylaxis and treatment for these conditions. Several American firms are now preparing serums containing antitoxin for *B. sordellii*.

#### SUMMARY

A case of chronic hypertensive cardiac disease with decompensation and multiple hemorrhages, in which there were a terminal peritonitis and septicemia due to invasion by *B. sordellii* from the intestinal tract, is described. This is believed to be the first recorded infection by *B. sordellii* without operative procedure or recent external trauma in man. It clearly indicates the occasional residence of this bacillus in the intestinal tract of man. This is also the fourth geographic locality to yield an infection with this organism. The propriety of including the antitoxin for *B. sordellii* in polyvalent serums for wound infections, peritonitis and intestinal obstruction is emphasized.

# General Review

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## AVIAN TUBERCLE BACILLUS INFECTION. WITH SPECIAL REFERENCE TO MAMMALS AND TO MAN

ITS REPORTED ASSOCIATION WITH HODGKIN'S DISEASE \*

ARNOLD BRANCH, M.D.

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Although Koch<sup>1</sup> was familiar with tuberculosis of birds, the probable specificity of the avian tubercle bacillus apparently did not at first suggest itself to him. At the Tenth International Congress on Medicine in Berlin, in 1890, however, from the evidence of Nocard,<sup>2</sup> Roux,<sup>1</sup> Rivolta,<sup>3</sup> and more especially Maffucci,<sup>4</sup> he declared that he was willing to accept the bacillus of tuberculosis of fowls as a separate species, though closely related to the mammalian type. He foresaw the important practical problem that would be presented, namely, the question of whether this organism was pathogenic for man, a problem which is not entirely solved today. As early as 1893, Kruse<sup>5</sup> stated that he had obtained cultures of avian tubercle bacilli from lesions in man and cattle and cultures of mammalian bacilli from the bodies of fowls, showing, in his opinion, that mammals were not always refractory to infection with the avian tubercle bacillus nor birds to infection with the mammalian type. In any discussion of this subject the papers of Koch and Rabinowitsch<sup>6</sup> and of Weber<sup>1</sup> on the specificity and identification of the organism should also be mentioned as pioneer work. The present review is attempted, and seems timely, for the reason that in recent years more and more interest has been shown in the pathogenicity of avian tubercle bacilli for mammals, as evidenced by the increasing num-

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\* From the Department of Pathology, Harvard Medical School.

1. Quoted by Calmette, A.: *Tubercle Bacillus Infection and Tuberculosis in Man and Animals*, tr. by W. B. Soper and G. H. Smith, Baltimore, Williams & Wilkins Company, 1923.

2. Nocard, E.: *Compt. rend. Soc. de biol.* **2**:601, 1885.

3. Rivolta, S.: *Gior. di anat., fisiol. e patol. d. animali* **21**:241, 1889.

4. Maffucci, A.: *Centralbl. f. Bakteriol.* **5**:237, 1889; *Centralbl. f. allg. Path. u. path. Anat.* **1**:409, 1890.

5. Kruse, W.: *Beitr. z. path. Anat. u. z. allg. Path.* **12**:544, 1892-1893.

6. Koch, M., and Rabinowitsch, L.: *Virchows Arch. f. path. Anat.* **190**:246, 1907.

ber of cases reported and by the campaign championed in this county by Van Es<sup>7</sup> for the eradication of tuberculous fowls. There have also entered medical literature reports of the possible etiologic connection of this organism with Hodgkin's disease (L'Esperance<sup>8</sup>), an observation, which, if confirmed, would be extremely important.

#### CHARACTERISTICS OF THE AVIAN TUBERCLE BACILLUS

It is necessary to define at the outset what requirements must be fulfilled if a diagnosis of infection with avian tubercle bacilli is to be made and to consider cases reported in the literature as authentic only when they meet these requirements. All others will be considered as probable but unproved, the evidence submitted being insufficient to warrant an absolute diagnosis.

Avian tubercle bacilli are acid-fast organisms that produce tuberculin or sensitize animals to a known tuberculin, preferably avian; as first pointed out by Straus and Gamaleia,<sup>9</sup> they grow more readily on ordinary solid culture mediums than the other types of tubercle bacilli. The growth is apt to be more moist and glistening than that of the mammalian type. On suitable fluid mediums (glycerin broth or synthetic mediums) growth occurs without the necessity of floating the inoculum on the surface, and a sediment as well as a surface pellicle forms. Avian tubercle bacilli are more viable in culture mediums than mammalian, and the optimum temperature for their growth is higher than that for the growth of the mammalian species (from 40 to 43 C.). Theobald Smith's acid curve resembles the bovine type (Bang<sup>1</sup>). Renfrew, Bass and Johnson,<sup>10</sup> using Long's synthetic medium at  $p_H$  6.6, found that in 4 weeks the  $p_H$  drops to 5.2 and then gradually rises in 16 weeks to the original, or a slightly higher  $p_H$ . Virulent avian tubercle bacilli are pathogenic for fowls and for rabbits after intravenous inoculation. In summary, the minimum requirements for a diagnosis of avian tubercle bacillus infection are acid-fastness with the capacity for forming tuberculin or for sensitizing animals to a known tuberculin, growth in suitable fluid mediums after inoculation in the depth of the medium and pathogenicity for fowls and rabbits after intravenous inoculation.

It is possible that the requirements may exclude some reported authentic cases of infection of man with avian tubercle bacilli. For

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7. Van Es, L.: J. Am. Vet. M. A. **76**:396, 1930.

8. L'Esperance, E. S.: J. Immunol. **16**:27, 1929; **16**:37, 1929; **18**:127 and 133, 1930.

9. Straus, I., and Gamaleia, N.: Arch. de méd. expér. et d'anat. path. **3**:457, 1891.

10. Renfrew, A. G.; Bass, S. L., and Johnson, T. B.: Am. Rev. Tuberc. **20**: 114, 1929.

example, Löwenstein was quoted by Krasso and Nothnagel<sup>11</sup> as saying that pathogenicity for chickens may be lost by passage through man. But until more definite methods are introduced for typing tubercle bacilli, one is not justified in including them. It must be remembered that there are cases of infection of man with acid-fast organisms not tubercle bacilli (Cobbett<sup>12</sup>). For example, Dr. Bayne-Jones has written me that he is at present studying such an organism grown from the purulent pleural exudate of a child. It is also possible that further study may place some of the cases reported here in this group.

Petroff and Branch<sup>13</sup> described 2 types of colonies from an avian culture. One of these was smooth and glistening and the other dry. Recently Petroff and Steenken<sup>14</sup> showed that the smooth "S" colony is virulent for rabbits and chickens, while the "R" colony in like doses is not. I have been able to isolate similar cultural dissociates from a strain obtained from L'Esperance. This work tends to throw some light on the variation in pathogenicity of avian strains and also on their occasionally atypical macroscopic appearances, cultures having been isolated that on solid mediums show a dry growth resembling that of mammalian strains. A purely pathologic anatomic diagnosis is not conclusive, even though, as will be seen later, the character of the lesion in birds is somewhat typical, and though Löwenstein<sup>15</sup> (1924) thought that there is a definite pathologic picture in man. Some cases of infection with avian tubercle bacillus in man are reported on this basis alone.

#### AVIAN TUBERCLE BACILLUS INFECTION OF BIRDS AND OF MAMMALS OTHER THAN MAN

*Birds.*—The following birds are susceptible to infection with avian tubercle bacilli: fowls, pigeons, pheasants, pea-fowls, turkeys, swans, canaries, finches, parrots, sparrows, arctic swans, vultures, ostriches, nandus, ibises, herons, gulls, eagles, crows, ducks and geese (Calmette<sup>16</sup>). Schalk<sup>17</sup> found it difficult to infect sparrows and pigeons by ingestion, although the disease occurs in them spontaneously. An interesting fact is that parrots, parrakeets and probably canaries are also susceptible to infection with mammalian tubercle bacilli.

11. Krasso, H., and Nothnagel, H.: Wien. Arch. f. inn. Med. **11**:507, 1925.

12. Cobbett, L.: Brit. M. J. **2**:255, 1918.

13. Petroff, S. A., and Branch, A.: Bull. Internat. A. M. Mus. **12**:135, 1929.

14. Petroff, S. A., and Steenken, W., Jr.: J. Exper. Med. **51**:831, 1930.

15. Löwenstein, E.: Ztschr. f. Tuberk. u. Heilstättenw. **7**:491, 1905; Wien. klin. Wchnschr. **26**:785, 1913; Ztschr. f. Tuberk. **41**:18, 1924; Med. Klin. **24**:1782, 1928.

16. Calmette, A.: Tubercle Bacillus Infection and Tuberculosis in Man and Animals, tr. by W. B. Soper and G. H. Smith, Baltimore, Williams & Wilkins Company, 1923.

17. Schalk, A. F.: J. Am. Vet. M. A. **72**:852, 1928.

The lesions of avian tuberculosis in birds are most common in the intestines and abdominal viscera. The intestines show ulcers and particularly encapsulated masses which project from the serous coats. The liver, spleen and abdominal lymph nodes are also studded with well demarcated, larger or smaller nodules. Histologically, acid-fast bacilli occur in very large numbers in the central vitreous or necrotic area, surrounded by mononuclears and foreign body giant cells arranged as a shell or sheath around conglomerate tubules. Besides this follicular or nodular form characterized by a granuloma with atypical necrosis and lack of caseation, of calcification and of typical Langerhans' giant cells, Chretien, Germain and Raymond<sup>18</sup> described a pseudoneoplastic form which resembles an encephaloid cancer and is composed of a proliferation of large polyhedral cells, with infiltration. Graduations between the 2 types occur.

*Mammals Other Than Man.*<sup>19</sup>—Spontaneous lesions have been reported in mice (de Jongh<sup>1</sup>), rats, rabbits, swine, cattle, sheep, horses, bullocks, monkeys, wallabies, and rat-kangaroos (Lucas<sup>20</sup>). Griffith<sup>21</sup> recently reported 2 cases of infection of the lymph nodes at the hilus in guinea-pigs exposed to tuberculous fowls. Guinea-pigs are generally considered resistant. Dogs are apparently not susceptible, though Feldman<sup>22</sup> reported dissemination after intracerebral inoculation. Mice and rabbits are susceptible. Guinea-pigs are resistant, except to large doses injected intraperitoneally, following which they succumb to septicemia. Plum<sup>23</sup> stressed the association of the avian tubercle bacillus with abortion of cattle. Swine are readily infected by feeding droppings of infected chickens (Schalk<sup>17</sup>). They do not, however, show generalized tuberculosis, the lesions being confined to the lymph nodes (Cornell and Griffith<sup>24</sup>). Schalk found that 88 per cent of localized tuberculous lesions in 30 swine selected at random in North Dakota and Western Minnesota were due to avian bacilli. Van Es and Martin<sup>25</sup> made a similar observation in Nebraska. Dr. Van Es<sup>26</sup> sent

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18. Chretien: Germain, and Raymond: *Rev. de la tuberculose* **4**:25, 1923.

19. For detailed studies see Griffith, F.: Report of the Royal Commission on Tuberculosis, Final Report, 1911, pt. 2, vol. 4, p. 167. Eastwood, A., and Griffith, F.: Report Local Government Board, no. 88, 1914. Griffith, A. S.: *J. Comp. Path. & Therap.* **38**:157, 1925.

20. Lucas, N. S.: *J. Path. & Bact.* **28**:123, 1925.

21. Griffith, A. S.: *J. Path. & Bact.* **33**:153, 1930.

22. Feldman, W. H.: *J. Am. Vet. M. A.* **76**:399, 1930.

23. Plum, N: *Senchenbekämpfung* **5**:62, 1928.

24. Cornell, R. L., and Griffith, A. S.: *J. Comp. Path. & Therap.* **43**:56, 1930.

25. Van Es, L., and Martin, H. M.: *Univ. Nebraska Coll. Agric. Exper. Sta. Research Bull.* 30, 1925.

26. Van Es, L.: Personal communication to the author; *J. Am. Vet. M. A.* **70**:775, 1927.

me an unpublished tabulation of the results of examination of 1,082 lesions from bovine, porcine, human and avian sources (table 1). By reference to table 1, it may be seen that avian tubercle bacilli were recovered from 11 of 164 isolated lesions in cattle, from 199 of 258 discrete lesions in swine, and from 104 of 110 lesions in birds. It was not discovered in 394 nonpulmonary lesions in man or in 3 cases of Hodgkin's disease. Plum<sup>23</sup> reported isolating both avian and bovine tubercle bacilli from a single cow. Cases of this sort may be missed if chickens, as well as guinea-pigs and rabbits, are not inoculated with

TABLE 1.—*The Distribution of Avian Tuberculosis in Mammals (Van Es)*

Material Used	Instances in Which the Different Types of Tubercle Bacilli Were Found in Lesions					Instances in Which Lesions Were Negative	Total
	Mammalian	Bovine	Human	Avian	Mixed		
Bovine: Isolated lesions, mostly of lymph nodes.....	100	..	...	11	4	49	164
Bovine: Lesions of skin.....	...	..	...	...	..	40	40
Bovine: Hemorrhagic lymph nodes (no lesions) .....	...	..	...	...	..	39	39
Bovine: Uteri (no lesions).....	...	..	...	...	..	5	5
Bovine: Generalized lesions.....	...	58	...	...	..	...	58
Swine: Chiefly lesions of isolated lymph nodes .....	12	..	...	199	14	33	258
Man: Lesions other than pulmonary....	...	11	216	...	..	167	394
Man: Hodgkin's disease.....	...	..	...	...	..	3	3
Sheep: Pulmonary lesions.....	...	1	...	...	..	...	1
Horse: Culture and lesions.....	...	2	...	...	..	...	2
Monkey .....	...	3	1	...	..	2	6
Fox .....	...	1	...	...	..	1	2
Pigeons, turkeys and fowls.....	...	..	...	104	1	5	110
Total .....							1,082

the original infected material or with different colonies of organisms isolated in culture.

For experimental inoculation the rabbit is the mammal most frequently used. Intravenous inoculation of large doses results in rapid death within 9 days, according to Parker and Spies,<sup>27</sup> while one is accustomed to seeing it occur in from 17 to 25 days. On the other hand, small doses administered intravenously are well borne, and the animals may survive and develop characteristic purulent joint lesions. Petroff and Steenken<sup>14</sup> showed that 0.3 Gm. of the "S" colony kills, while similar doses of the "R" colony are well borne. Subcutaneous inoculation of some strains may kill (Lucas<sup>20</sup>). It is customary to speak of the former type as death from bacilleemia of Yersin, though in my experience histologic examination of the spleen, bone marrow and liver yields evidence of typical formation of tubercles and, with the more virulent bacilli, areas of infarction and necrosis.

27. Parker, F., Jr., and Spies, T.: Personal communication to the author.



*Summary.*—In this preamble it has been pointed out that the avian tubercle bacillus is the causative agent in the greatest number of cases of tuberculosis in birds, but that apparently some of these are relatively immune. Some birds, notably the parrot, may be readily infected also with mammalian types. In the case of mammals other than man, swine show the greatest incidence of avian tuberculosis; it also occurs in cattle. Experimentally, mice and rabbits may be infected.

#### AVIAN TUBERCLE BACILLUS INFECTION IN MAN

*Clinical Manifestations.*—Table 2 shows briefly the lesions that have been reported. From the table it may be seen that the disease manifests itself in various forms, the most common being ulcers and abscesses of the skin and mucous membranes, renal tuberculosis, a septic form simulating typhoid fever, "sarcoid" of the skin, dyscrasia of the blood and enlargement of lymph nodes. The point stressed by most authors is the occurrence of a specific reaction to avian tuberculin in much higher dilution than that of mammalian tuberculin.

Löwenstein,<sup>15</sup> in discussing cases of infection with avian tubercle bacillus in man, stated that the first stage is accompanied by a long continued remittent fever, with elevation of the temperature in the evening. A splenic tumor is almost always present. There are three sites of predilection for metastases: (1) the bone marrow, (2) the kidney and (3) the skin. To these should be added the lymph nodes.

Cutaneous Lesions: Urbach<sup>28</sup> spoke of 4 different types of lesions of the skin; he considered the first 3 of these to be hematogenous and the fourth to be due to direct inoculation: (1) an aphthous septicemic form with changes in bones and joint, (2) a sarcoid-like lesion, (3) a gummatous form and (4) a local ulcerous form.

The ulcers tend to heal and recur. The lesions may be mistaken for those of syphilis, atypical tuberculosis, sporotrichosis or even leprosy.

Urogenital Form: The interesting point in the cases of urogenital infection is the rapid disappearance of the bacilli following treatment with avian tuberculin, some cases having been followed for 6 years and longer. In 1 of Joannovic's<sup>29</sup> cases "healing" was apparently spontaneous. In Deutsch's<sup>30</sup> case, the kidney was removed and showed extensive disease with cavitation. In spite of the long duration of the infection, the urinary bladder is usually not involved or but slightly. The bacilli are apt to be intracellular (polymorphonuclear leukocytes) and to occur in very large numbers.

28. Urbach, E.: Arch. f. Dermat. u. Syph. **157**:360, 1929.

29. Joannovic, G.: Wien. med. Wchnschr. **73**:22, 1923.

30. Deutsch, I.: Med. Klin. **21**:1883, 1925.

Lesions of the Bone Marrow: Little at present is known as to the lesions of the bone marrow, except that in 2 cases of clinical "polycythemia rubra" and in 5 of "myelogenous leukemia" avian tubercle bacilli were found post mortem (quoted by Löwenstein<sup>15</sup>). Elias and Hitzengerger<sup>31</sup> observed that the splenic tumor in such cases is not susceptible to roentgen treatment.

That all cases of polycythemia or leukemia are not necessarily due to this organism is suggested by the careful work of Pinkerton<sup>32</sup> on several cases of an unusual leukemic condition in children. He was unable to demonstrate acid-fast bacilli in sections in any of his cases.

*Pathologic Anatomy.*—Pathologic material to date is rather meager, consisting of some half dozen autopsies (Löwenstein,<sup>15</sup> L'Esperance<sup>8</sup> and others). Erdheim was credited by Gasul<sup>33</sup> with having studied 2 other cases. The autopsies are summarized individually in later paragraphs, with the histories of the cases. Besides these, 1 surgically removed kidney was described by Deutsch<sup>30</sup> and biopsies of skin by other authors.

Spleen: As stated in the clinical discussion of these cases, a sepsis followed by localization in one or more sites (i.e., bone marrow, kidneys, skin, etc.) is the rule, and evidence in support of these clinical symptoms is found. Thus the spleen was enlarged (260 Gm. and over) at all autopsies. It may be hard or soft, depending on the duration of the disease. There may be no other gross lesions in this organ, or there may be necrosis without caseation. This picture is claimed to be histologically characteristic. There is practically no surrounding cellular reaction. Tubercle bacilli are reported in the lesion in some instances but particularly in the surrounding tissue. I have had the opportunity of studying the sections of this interesting type of necrosis from L'Esperance's case. They remind one of the necrotic areas seen in the spleen of a guinea-pig dying of fulminating tuberculosis. In Dugge's<sup>34</sup> case (classed here with the probable group) the foci in the spleen were composed of poorly defined cellular aggregations with much nuclear debris.

Kidneys: Deutsch<sup>30</sup> reported caseous tuberculosis in a surgically removed kidney. The kidneys were involved in two autopsies reported. Grossly the lesions appeared as small, punctiform, abscess-like foci in the cortex and medulla. Histologically these areas were composed of polymorphonuclear cells and debris, with acid-fast bacilli in large num-

31. Elias and Hitzengerger, quoted by Löwenstein (footnote 15, third reference).

32. Pinkerton, H.: Aleukemic Leukemia and Atypical Leukemoid Conditions: Report of Seven Cases Including One of Acute Erythroblastosis, Arch. Path. 7:567, 1929.

33. Gasul, B. M.: Arch. Pediat. 46:67, 1929.

34. Dugge, M.: Beitr. z. Klin. d. Tuberk. 71:538, 1929.

bers, many situated within the phagocytic cells. In 1 case vascular changes comparable with those seen in tuberculous meningitis were also reported.

**Bone Marrow:** In 3 cases reported in which the bone marrow was involved, no tubercles were found. L'Esperance described necrosis, giant cell formation and granulomatous tissue. In the other 2 cases there was fibrosis.

**Lungs:** Löwenstein described multiple pea-sized, regular grayish foci in the lungs. These foci were composed of an intra-alveolar exudate of leukocytes and red blood cells. L'Esperance's case showed nodular areas of consolidation like an interstitial pneumonia.

**Liver:** The liver may be enlarged and show either tubercles or stippling corresponding to small foci of necrosis.

**Lymph Nodes:** The lymph nodes were prominently involved and especially attracted attention in 2 cases (Dugge<sup>34</sup> and L'Esperance<sup>8</sup>). Dugge reported hilar and mesenteric lymph nodes, each varying in size from that of a plum to that of a hazelnut, which were composed of poorly defined cellular aggregations with central areas of nuclear débris. These often fused to form larger areas. In L'Esperance's case, there was a chain of nodes in the neck which extended down around the aorta. I have examined these nodes; the picture is that of a non-specific granulomatous chronic inflammatory process. Enlarged cervical lymph nodes were also observed in the case of Kerl<sup>35</sup> and of Urbach,<sup>28</sup> in which there was an ulcer at the base of the tongue.

**Skin and Mucous Membranes:** Urbach described 4 types of avian tuberculosis of the skin: an aphthous septicemic form, with changes in bones and joint, a gummatous form, a sarcoid and a local exogenous ulcerous form. The first 3 he considered to be hematogenous.

The aphthous septicemic form is illustrated in the case reported by Kerl and Urbach independently. There were superficial ulcers of the tongue, scrotum and mucosa of the lips, tongue and pharynx. The ulcers were not typical of tuberculosis, and acid-fast bacilli were not demonstrated, but avian tubercle bacilli were grown from the tissue. The ulcers tended to heal and reappear, or others developed. There was little or no scarring.

The gummatous type is illustrated by Lipschutz's<sup>36</sup> case. Ulcers had appeared 10 years previously on the soft palate, hard palate, nose and trunk. There was induration of the upper lip, and an ulcer occurred, with sharply demarcated edges and a base covered with red, suppurative granulations. The skin showed multiple brownish-red, nodular infiltrations, slightly elevated, round and plateau-like, with occasional ulcera-

35. Kerl, W.: *Wien. med. Wchnschr.* **78**:840, 1928.

36. Lipschutz, B.: *Arch. f. Dermat. u. Syph.* **120**(O.):387, 1914.

tion. Histologically there was a chronic diffuse inflammatory granulation tissue, consisting of epitheloid cells, round cells and rarely tubercle-like foci with no caseation. Multiple subcutaneous abscesses developed later. These ruptured spontaneously and discharged a thick, creamy pus containing large numbers of acid-fast bacilli. The abscesses differed from the ordinary cold abscess in developing in a few days, with intense pain, fever and redness of the skin.

In summary, Löwenstein stressed the enlargement of the spleen, the involvement of the kidney and bone marrow and a histologic picture that differs from the usual picture of tuberculosis in (1) the presence of disseminated foci in the kidney similar to abscesses, (2) the presence of an exudate in the pulmonary alveoli consisting of polymorphonuclear leukocytes, (3) the complete absence of proliferative changes and caseation, (4) the presence of necrosis in the spleen and (5) the large number of tubercle bacilli in the foci, their situation being chiefly intracellular. Involvement of the lymph nodes is also encountered.

#### REPORTED CASES OF AVIAN TUBERCLE BACILLUS INFECTION IN MAN

CASE 1 (Löwenstein,<sup>15</sup> 1905).—A man's sputum contained acid-fast bacilli with characteristics of the avian tubercle bacillus. A pure culture was sent to Rabinowitsch and Weber, who confirmed the diagnosis.

CASE 2 (Rabinowitsch,<sup>37</sup> 1907).—Avian tubercle bacilli were isolated from the spleen of a man aged 21. (We have been unable to obtain the original report of this case, which was referred to by Löwenstein as authentic.)

CASE 3 (Jansco and Elfer,<sup>38</sup> 1910).—Avian tubercle bacilli were isolated from the tuberculous mesenteric lymph nodes of a girl aged 8.

CASE 4 (Löwenstein,<sup>15</sup> 1913).—A girl, aged 3, had fever for about 2 years, the temperature rising in the evening to 39 C. (102.2 F.). Otherwise she was in good condition, except for night sweats. There was no physical or roentgenologic evidence of pulmonary tuberculosis. The urinary sediment contained many acid-fast bacilli. She did not react to human tuberculin; to avian tuberculin, even in a dilution of 1:100,000, there was an intense focal, local and general reaction. Rabbits inoculated intravenously with the urinary sediment died in from 4 to 6 weeks, and acid-fast bacilli were grown from the heart's blood. Chickens inoculated intravenously died with circumscribed tuberculosis in from 4 to 6 months.

CASE 5 (Löwenstein,<sup>15</sup> 1913).—A boy, aged 12, presented a picture very similar to that in case 4, except that acid-fast bacilli were found in the kidney only after injection of avian tuberculin. The animals inoculated with urinary sediment reacted as in case 4.

CASE 6 (Löwenstein,<sup>15</sup> 1913).—Multiple infiltrations of the skin and subcutaneous abscesses with ulcers in the nose, mouth and intestine marked case 6. Large numbers of intracellular acid-fast bacilli were found, suggesting leprosy. Chick-

37. Rabinowitsch, L., quoted by Löwenstein (footnote 15, second reference).

38. Jansco, N., and Elfer, A., quoted by Löwenstein (footnote 26, second reference).

ens inoculated with the organism died with tuberculosis in 7 months. The organism was not very pathogenic for rabbits or for guinea-pigs.

CASE 7 (Lipschutz,<sup>36</sup> 1914).—A man, aged 20, had suffered since 1904 from ulcerous processes of the soft palate and 2 years later from similar changes in the hard palate and in the skin of the nose. The patient was poorly nourished, without obvious disease of any internal organ. The upper lip was indurated and was twice the normal size and ulcerated. The base of the ulcer was red and the edge sharp, and it extended into the nose. The uvula was absent; the right palatoglossus was adherent to the posterior wall of the pharynx; the gums were infiltrated, and there was an ulcer in the middle of the hard palate. In 1908, multiple lesions of the skin developed. These were elevated, flat, brownish-red nodules, covered with epithelium and occasional small ulcerations. They were well outlined, were round or lobulated, and varied in size from 1 to 2 cm. Some of these nodules later involuted and left dirty yellowish, atrophic scars. Still later there was an afternoon rise in temperature, and large subcutaneous abscesses developed, which ruptured and discharged yellow pus and healed by granulation tissue. The pus contained acid-fast bacilli, which produced disease in chickens and not in guinea-pigs.

CASE 8 (Joannovic,<sup>29</sup> 1923).—A woman in her twenties had bronchitis and a temperature of 102.2 F. and later a tumor, the size of a fist, under the left costal arch. After several months the urine became clouded, and contained polymorphonuclear leukocytes and a large number of acid-fast bacilli. Guinea-pigs inoculated intraperitoneally did not become infected, but chickens inoculated intraperitoneally with urinary sediment died of progressive emaciation and tuberculosis. The patient was treated with avian tuberculin; the temperature declined, and tubercle bacilli disappeared from the urine in a year. Six years elapsed, and the patient was still well, except for bronchitis and slight elevation of temperature, but acid-fast bacilli could not be demonstrated by inoculation of either the sputum or the urine into animals.

CASE 9 (Joannovic,<sup>29</sup> 1923).—In a voluntary war nurse in her middle twenties, who previously had been in charge of a chicken house, an evening temperature of 102.2 F. developed, which did not improve in a mountain climate and with sun baths. Later pus developed in the urine, with colon bacilli. Joannovic, however, later found acid-fast organisms in large numbers in the urinary sediment. This was inoculated into guinea-pigs and chickens intraperitoneally. The chickens died of tuberculosis, and the guinea-pigs remained well. The patient improved spontaneously, and six years after the onset of her disease appeared entirely well.

CASE 10 (Deutsch,<sup>30</sup> 1925).—A woman, aged 57, one of whose sisters had pulmonary tuberculosis, had, at the age of 14, a mass in the right axilla, which was extirpated when she was 28. Seventeen years later a new tumor developed at the same site. Ten years before examination laryngitis set in, and the patient had been hoarse since. For over a year she had had pains in the region of the kidney and pain and tenesmus during urination. The region of the left kidney was sensitive to palpation. The axillary lymph nodes were extirpated and histologically showed tuberculosis. Cystoscopic examination revealed pus from the left ureter; the urinary sediment repeatedly showed acid-fast bacilli. Culture on glycerin potato yielded a yellow, moist, confluent growth. Inoculations were made into guinea-pigs, rabbits and fowls. The guinea-pigs remained healthy; the 2 rabbits died in 3 weeks with sepsis; the chicks, sick after 4 months, were killed and examined and showed tuberculosis of the internal organs. The patient's kidney was removed and showed a tuberculous cavitation.

CASE 11 (Kerl and Urbach independently, 1925).—A Ukrainian man, aged 31, was suddenly attacked by chills and fever, with cough, and a stabbing pain in the sternal region,  $1\frac{1}{2}$  years before examination. On examination the patient was greatly emaciated and presented at the junction of the left rib and sternum a painful region, which on roentgen examination did not show lesions. There was dulness at the apex of a lung, but the x-ray picture revealed active tuberculosis. Several ulcers were present on the tongue, both lips and scrotum. They tended to heal and recur. Under observation pain and swelling occurred in the knee joint, with acne-like lesions on the forehead, nose and cheeks, while grave cachexia and evening fever persisted. Over a year later new ulcers appeared on the palate and lips. Intracutaneous inoculation of old tuberculin in a dilution of 1:10,000 elicited a slight reaction, but that of avian tuberculin was followed by an extensive reaction and ulceration. No acid-fast organisms were found in smears of the ulcers, but were cultivated. These were pathogenic for guinea-pigs, rabbits (on intravenous and intra-ocular inoculation) and fowls.

CASE 12 (Volk quoted by Urbach <sup>28</sup>).—In a poultry worker, 12 years previous to examination, an ulcer of the left leg developed. There was a history of injury to the left external malleolus while the patient was walking barefoot. An intracutaneous test with old tuberculin in a dilution of 1:1,000 resulted negatively, while the reaction to avian tuberculin in a dilution of 1:10,000 was strongly positive. Bacilli were not demonstrated in the sections, but Löwenstein grew typical avian tubercle bacilli.

CASE 13 (Knossew quoted by Urbach <sup>28</sup>).—In a farm worker, 10 years before the illness in question, abscesses developed on the right leg, which repeatedly healed and broke. He reacted to avian tuberculin in a dilution of 1:1,000,000, and much less strongly to old tuberculin. Löwenstein cultivated avian tubercle bacilli. The lesions decreased under treatment with avian tuberculin.

CASE 14 (L'Esperance, <sup>8</sup> 1930).—This case of Murchison-Pel-Epstein syndrome was reported as additional evidence in favor of the etiologic agent of Hodgkins' disease being related to the avian tubercle bacillus, 3 cases of Hodgkins' disease having previously been reported by L'Esperance. After examination of sections sent to me, I feel that a diagnosis of Hodgkins' disease cannot be made on two lymph nodes and the spleen; I regard this as a true case of infection with avian tubercle bacillus in man, the first to be reported in the English language.

*History.*—A man, aged 25, who came from the West Indies, noticed an enlarged lymph node on the left side of the neck  $1\frac{1}{2}$  years previous to examination. It did not increase in size. For  $2\frac{1}{2}$  months before his entrance into the hospital he had recurring attacks of chills, with a temperature of between 98 and 104 F. and loss of weight. Physical examination revealed a chain of small, discrete cervical nodes on the left, small nodes in the axilla and groin and a spleen palpable 4 fingerbreadths below the costal margin. "The blood count was not distinctive." An x-ray picture revealed enlarged nodes at the hilus of the lungs.

*Autopsy.*—Postmortem examination showed scanty, flat, reddish macules in the skin over the chest. In the lower lobe of the left lung were nodular areas of consolidation and fibrous adhesions. The right lung was pneumonic, there being numerous dense, dark red areas. The bronchial and superficial nodes were moderately enlarged. The spleen was greatly enlarged and firm, and showed numerous opaque, tumor-like nodules. The liver was large and irregularly congested, with a smooth, waxy surface. Histologically the spleen revealed numerous discrete and conglomerate pinpoint-sized to pea-sized focal areas of necrosis, with

hemorrhages over the whole surface. There was little cellular reaction of the type of Hodgkins' granuloma, but rather typical myeloid giant cells. The lungs were congested, the alveoli were filled with pigmented cells, and around clusters of congested alveoli the septums showed a peculiar interstitial pneumonia, the cellular overgrowth being mainly of epithelial cells. The bone marrow in the lumbar vertebrae showed necrosis, with myeloid giant cells and a deposit of granulomatous tissue. Direct smears of the spleen showed a few acid-fast bacilli and cultivation on egg mediums yielded a scant, moist growth resembling that of the avian tubercle bacillus.

*Experimental Results.*—Macerated spleen was inoculated into 4 chickens intravenously (these were also fed splenic pulp), 1 rabbit intravenously, 1 rabbit subcutaneously, 2 normal guinea-pigs and 2 guinea-pigs that previously had been inoculated with heat-killed mammalian tubercle bacilli. One of the chickens, killed after 4 months, showed early tuberculous lesions in the liver, spleen and lymph nodes. Acid-fast granules were found in stained sections. A second chicken, killed and examined after 6 months, showed extensive tuberculosis of the liver and spleen. Acid-fast organisms were cultivated on egg medium, the growth being similar in appearance to that obtained from the spleen originally. No further mention is made of the remaining 2 chickens. The intravenously inoculated rabbit died in 20 days, with acute fibrinous pleuritis, small focal areas in the liver, consolidation of the caudal lobes of both lungs and a large node in the left groin. Cultures from the nodules in the liver on egg medium showed a moist growth of acid-fast bacilli with many cultural characteristics of the avian type. Macerated tissue from the nodes of this rabbit was inoculated intravenously into another rabbit, which died in 22 days with acute septicemia, foci in the liver and spleen and cheesy pleurisy. Cultures from the pleural exudate on hormone agar and broth were negative, while on egg medium they gave a moist growth of acid-fast bacilli. The second rabbit inoculated subcutaneously with the patient's splenic tissue lived 6 months and at autopsy showed generalized tuberculosis. One of the "treated" guinea-pigs died 11 months after inoculation, with extensive tuberculosis of the lymph nodes, slight involvement of the lungs and a single focus in the liver. The other 3 were alive 372 days after inoculation.

COMMENT.—Besides the cases mentioned, Löwenstein<sup>15</sup> referred to 1 case of polycythemia rubra and 4 cases of myelogenous leukemia from the lesions of which at autopsy avian tubercle bacilli were isolated. Gasul<sup>33</sup> also quoted a personal communication from Professor Erdheim of Vienna in which he stated that he had performed autopsies on 2 patients with avian tuberculosis. The bones were especially affected, showing necrosis and being filled with avian tubercle bacilli. Graham<sup>39</sup> reported finding an "aberrant" strain slightly pathogenic for fowls along with an organism of mammalian type in a case of cervical adenitis and in 17 of 115 tuberculous sputums.

Rabinowitsch-Kempner<sup>40</sup> further quoted the typing of 1,861 cases of human tuberculosis by Möllers among which there were 3 with avian organisms.

In his last paper Löwenstein called attention to a report of the Egyptian Board of Health in which urogenital tuberculosis is referred to as the "true plague of Egypt." The frequency of this form of tuberculosis was traced to the fact that the natives live in the same room with their poultry. Löwenstein also quoted a personal communication from Dr. Brown stating that urogenital tuberculosis is common in the Philippines, where the same custom exists.

39. Graham, R.: Illinois M. J. **50**:210, 1926.

40. Rabinowitsch-Kempner, L.: Am. Rev. Tuberc. **15**:225 and 419, 1927.

REPORTED CASES OF PROBABLE AVIAN TUBERCLE BACILLUS  
INFECTION IN MAN

These include all cases in which sufficient data are not supplied to enable one to be certain of the identity of the acid-fast organism.

In this group should be mentioned, as pointed out by Löwenstein (1928), the cases published by Landouzy<sup>41</sup> in 1891. He described several cases in which there were noted malaise, enlarged spleen and remittent fever of the typhoid type, but

TABLE 2.—*Reported Infections with Avian Tubercle Bacillus in Man*

Authors *	Year	Sex and Age of Patient	Outstanding Clinical Picture	Reaction to Tuberculin	
				Avian	Human
Löwenstein <sup>15</sup> .....	1905	M	Pulmonary involvement	.....	..
Rabinowitsch <sup>37</sup> .....	1907	..	Splenic involvement	.....	..
Janseo and Elfer <sup>28</sup> .....	1911	..	Lesions of mesenteric lymph node	.....	..
Löwenstein <sup>15</sup> .....	1913	F 3 yr.	Evening fever; renal tuberculosis	+ to dilution of 1:100,000	—
Löwenstein <sup>15</sup> .....	1913	M 12 yr.	Evening fever; renal tuberculosis	+ to dilution of 1:100,000	—
Löwenstein <sup>15</sup> .....	1913	..	Cutaneous abscesses; ulcers of nose, mouth and intestine	.....	..
Lipschutz <sup>36</sup> .....	1914	M 20 yr.	Ulcers of palate and of skin of nose	.....	..
Joannovic <sup>29</sup> .....	1923	F 20 yr.	Fever; renal tuberculosis; tumor of skin	.....	..
Joannovic <sup>29</sup> .....	1923	F 25 yr.	Fever; renal tuberculosis	.....	..
Deutsch.....	1925	F 57 yr.	Tumor of axilla; renal tuberculosis	.....	..
Urbach <sup>28</sup> and Kerl <sup>25</sup> .....	1927 1928	M 32 yr.	Septic fever; ulcers of tongue, lips and scrotum; swelling of knee joint	+ to dilution of 1:10,000	—
Volk (quoted by Urbach <sup>28</sup> )....	1929	M	Ulcer of leg	+ to dilution of 1:10,000	—
Knosew (quoted by Urbach <sup>28</sup> )	1929	M	Abscesses of leg	+ to dilution of 1:100,000	±
L'Esperance <sup>8</sup> .....	1929	M 25 yr.	Septic fever; Pel-Epstein's syndrome	.....	..

\* Löwenstein quotes reports on avian tubercle bacilli in 4 cases of myelogenous leukemia and 1 of polycythemia rubra; Erdheim (quoted by Gasul<sup>22</sup>) performed autopsies in 2 cases.

+ = Positive reaction in dilution given.

— = Negative reaction.

in which neither typhoid fever nor tuberculosis could be proved. The patients who came to autopsy did not show lesions of typhoid fever or gross evidence of tuberculosis, except enlarged spleens containing grayish nodules in which, histologically, acid-fast bacilli were demonstrated. Three so-called cases of "typhobacillose—Landouzy" were reported by Neuman, Krokiewicz, Reiche and Scholz.<sup>41</sup>

41. Landouzy, Neuman, Krokiewicz, Reiche and Scholz, quoted by Löwenstein (footnote 15).



Pansini,<sup>42</sup> in 1894, recovered from two guinea-pigs inoculated with tuberculous material from man acid-fast organisms that culturally resembled the avian tubercle bacillus. Nocard,<sup>43</sup> in 1898, inoculated tuberculous sputum into rabbits and guinea-pigs. The rabbits were killed rapidly, while the guinea-pigs survived and at autopsy showed lesions that resembled those of avian tuberculosis. Reed,<sup>44</sup> in 1902, reported a case with large areas of necrosis in the spleen, in which tubercle bacilli were found.

In 1922, Lederer<sup>45</sup> reported a case of "avian tuberculosis." A woman, aged 49, had an enlarged liver and spleen. Her blood presented the picture of polycythemia. At autopsy two surprises were encountered: an absence of the marked congestion of organs seen in polycythemia and an absence of erythropoiesis of the marrow of the long bones. Tuberculosis of the lungs, spleen and kidneys was found. Histologically, many acid-fast bacilli were found in the lungs, kidneys, spleen and liver, but the histologic appearances were atypical. The lung showed caseous pneumonia, with preponderating polymorphonuclear leukocytes, and no caseation. In the kidneys were abscess-like foci. The spleen showed necrosis, with no tubercle bacilli in these areas, but they were present in the surrounding tissue. Miliary tubercles occurred in the liver, and the bone marrow showed blood-forming foci, with fibrosis, but no tuberculous lesions. The author thought that it resembled tuberculous bacteremia of a peculiar form, probably due to the avian type.

In 1925, Krasso and Nothnagel<sup>46</sup> reported a case of "atypical (avian?) tuberculosis in myeloid leukaemia." A man, 45 years old, observed over a period of years, had myeloid leukemia and no evidence of tuberculosis. At autopsy numerous tubercles the size of hemp seeds were found in the lung, lymph nodes and spleen. The tubercles contained massive numbers of acid-fast organisms and histologically did not present the typical picture of tuberculosis in man. Cultures produced a moist growth, and the organisms were pathogenic for guinea-pigs and rabbits, but not for pigeons and chickens. After passage through an animal the colonies showed dry growth.

Although in cultural characteristics this organism resembled the avian bacillus, its pathogenicity for animals would place it in the bovine group. The work of Petroff showed that tubercle bacilli dissociate and form atypical "moth ball" colonies, and Dr. Aronson showed me a culture which resembled the avian type, but which in pathogenicity fell into the bovine group."<sup>46</sup> Löwenstein, however, apparently accepts this case as authentic.

Two cases were reported from the Mayo Clinic in 1926 (Mayo and Hendricks<sup>47</sup>), but the diagnosis was made on surgical and pathologic evidence alone. In the first case, a woman, aged 26, had secondary anemia and an enlarged spleen. The differential blood count showed 4.5 per cent mononuclears, 4 per cent transitionals and 9 per cent eosinophils. On physical examination the chest appeared to be normal, and no acid-fast bacilli were found in the sputum. Gastric

42. Pansini, S.: *Deutsche med. Wchnschr.* **20**:694, 1894.

43. Nocard: *Congress pour l'étude de la tuberculose* **4**:661, 1898.

44. Reed, D. M.: *Bull. Johns Hopkins Hosp.* **10**:133, 1902.

45. Lederer, K.: *Wien. Arch. f. inn. Med.* **5**:23, 1922.

46. Aronson: Personal communication to the author.

47. Mayo, C. H., and Hendricks, W. A.: *South. M. J.* **19**:29, 1926.

analysis showed a total acidity of 20 and no free hydrochloric acid. A splenectomy was performed, and the patient convalesced uneventfully. At operation the spleen was found to be large and nodular, and the liver was covered with yellow spots exactly as seen in chickens and turkeys with tuberculosis.

In the second case, a woman, aged 22, complained of pain beneath the lower end of the sternum and a mass in the abdomen of three months' duration. Examination revealed a large, but not tender, spleen. The hemoglobin content was 72 per cent and later 61 per cent. A differential white blood cell count showed 2 per cent large mononuclears, 4 per cent transitionals and 1 per cent eosinophils. A splenectomy was performed. The spleen was 27 by 15 by 7.5 cm., and the pathologist's report was: "The external surface of the spleen presented numerous nodules varying from 1 mm. to 2 cm. in diameter. These nodules (conglomerate tubercles) bear a close resemblance to the cortical abscesses of acute purulent nephritis. On section the nodules or tubercles stand out in relief, giving the surface a 'pebble dash' effect. Foreign body giant cells are present in some areas. A number of tubercles contain a waxy substance instead of the caseous material that is often found in tubercles in cases of tuberculosis in man. In many tubercles there is no caseation; the center is composed of large epithelioid cells in great numbers, which give a characteristic appearance to the avian lesion, in contrast to that of human and bovine tuberculosis. Lymphocytes are relatively less numerous than in lesions in man." The patient left the hospital on the twentieth day after operation.

Urbach<sup>28</sup> in 1929 reported two cases of sarcoid, the basis for the diagnosis being a stronger reaction with avian than with mammalian tuberculin. In the first case, a woman, aged 23, more than three years before examination was under treatment for infiltration of the cheek; the lesion was bluish red, with the histologic picture of Darier-Roussy sarcoid. On examination the left cheek was markedly atrophied. On the upper part of the right arm was a bluish-violet infiltration the size of a five crown piece, adherent to the underlying tissues, hard and seemingly the size of a small apple. The results of clinical roentgen examinations were negative. The result of an intracutaneous test with old tuberculin in a dilution of 1:50,000 was negative, but the same dilution of avian tuberculin gave a markedly positive reaction. Six weeks after this there was a slight reaction to old tuberculin in a dilution of 1:10,000 and a stronger reaction to avian tuberculin in the same dilution. The bluish-violet nodule in the skin had completely disappeared.

The second case reported by Urbach was similar to the first, there being sarcoid-like tumors (histologically considered) and a stronger reaction to avian tuberculin than to old tuberculin. The patient improved under treatment with avian tuberculin.

The final case, one of typhoid-like sepsis due to an acid-fast bacillus not pathogenic for guinea-pigs, was reported by Dugge<sup>34</sup> in 1929. Pathologically the evidence favored a diagnosis of infection with avian tubercle bacillus. A man, aged 65, was admitted to the hospital in a state of stupor, with a history of eight days' illness beginning with a high fever, stupor, pain in the extremities and neck and headache with occasional profuse diarrhea. On examination a stuporous, restless patient was seen, with cyanosis and incontinence of urine. There was some dyspnea; the lower part of the chest was somewhat dull to percussion, and in this area moist râles and bronchovesicular breathing were present. The heart was rapid and irregular, with a basal systolic murmur. The pulse was small; the abdomen was distended and diffusely tender. No splenic or hepatic enlargement was demonstrated until three days later when the spleen was definitely palpable.

The specific gravity of the urine was 1.023, and there was a trace of albumin. The sediment contained hyaline casts and some red blood cells. The temperature remained high. The leukocyte count was 8,360. No typhoid bacilli were found in the stool, urine or blood, and the result of the Widal test was negative. The patient died in a few days with a diagnosis of typhoid fever.

At autopsy, the observations positive for infection with tubercle bacilli were: The spleen weighed 260 Gm.; it was firm and dark red, with prominent follicles and white spots the size of a cherry on the cut surface and under the capsule. The coronary arteries showed hardened walls. The lungs were dark red and anthracotic, with patches of consolidation. In the lower right pulmonary artery were friable red clots. The lymph nodes at the hilus were each the size of a hazelnut, firm and dark grayish red. Each of the mesenteric nodes was the size of a plum, firm and of an even yellow consistency. The liver presented subcapsular needlepoint-sized white stipplings, also seen in cut surface.

On microscopic examination, the foci in the mesenteric and bronchial nodes, the spleen and the liver showed much the same histology and contained numerous acid-fast bacilli. The size of the foci ranged from that of 1 to that of 5 lymph follicles, the larger being formed from the confluence of the smaller ones. The center was composed of a few small fibrinous strands and cellular detritus, while the periphery showed cells with large, oval, vesicular nuclei and the fine reticulum of the node. Cultures from the spleen and the liver yielded a rapidly growing, acid-fast organism, not pathogenic for guinea-pigs and mice.

Rulison<sup>48</sup> reported two cases with cutaneous lesions (Hodgkin's disease and lupus erythematosus) in which the reaction to avian tuberculin was more marked than that to mammalian tuberculin.

#### OTHER UNUSUAL GRANULOMAS POSSIBLY DUE TO INFECTION WITH THE AVIAN TUBERCLE BACILLUS

*So-Called Boeck's Sarcoid.*—In 1899, Boeck<sup>49</sup> reported one case and in 1900 three cases of a peculiar nodular condition of the skin, particularly of the face, the etiology of which was obscure. Since then many cases have been reported, the etiology being still obscure, with opinions leaning to syphilis, to an unusual form of tuberculosis or to a combination of both. Urbach reported two cases of sarcoid in which the patients reacted more strongly to avian tuberculin.

Briefly, the lesions consist of chronic small or large subcutaneous nodules, varying in number from one to hundreds, situated usually in the face, but also on the extremities and trunk. The nodules require months or years for full development, and are bluish red or brownish red, the color at the periphery fading later to a yellowish brown. They may involute and leave pigmented, atrophic scars. One may recall that this description is not unlike that quoted by Lipschutz of a condition due to infection of the skin with avian tubercle bacillus, except that ulceration does not usually occur and acid-fast bacilli are not demonstrable, certainly not in the large numbers reported by Lipschutz. The lesions are made up of isolated small tubercles composed of mononuclear cells and separated by fibrous tissue. Caseation does not occur. Recently a case of this description was reported (Bernstein, Konzelmann and Sidlick<sup>50</sup>) with visceral lesions in the epicardium, in

48. Rulison, R. H.: Arch. Dermat. & Syph. **21**:901, 1930.

49. Boeck, C.: J. Cutan. & Genito-Urin. Dis. **17**:543, 1899.

50. Bernstein, M.; Konzelmann, F. W., and Sidlick, D. M.: Arch. Int. Med. **44**:721, 1929.

the wall of the intestine and around the bronchi. Tissue excised in a case of Boeck's sarcoid at the Peter Bent Brigham Hospital, recently studied by the author with Drs. Wolbach and Schulz, failed to produce lesions in guinea-pigs, rabbits, mice, monkeys and fowls.

*Other Obscure Granulomas.*—Wolbach<sup>51</sup> described cases in which similar granulomas occurred in the spleen, lymph nodes, liver and lungs, characterized by the presence of large, star-shaped inclusion bodies in the cytoplasm of the giant cells. No definite opinion can at present be given on the etiologic factor involved here.

#### METHOD OF INFECTION IN MAN

All observers agree that, excluding the localized lesions of the skin, the mode of entry is by food, and the question of importance concerns the source of the infection. Infection would most likely occur through the eating of infected organs of fowls or of infected eggs. Since in countries where human infection with avian tubercle bacilli has been reported, fowls are cooked, and no evidence is at hand to support infection from this source, one must focus attention on the occurrence of infected eggs, which are often eaten after insufficient heating to kill all the tubercle bacilli that may be contained in them. Löwenstein demonstrated tubercle bacilli in eggs removed from the cloacas of tuberculous hens and showed that eggs artificially infected with avian tubercle bacilli and boiled for only a few minutes still harbor living bacilli. Raebiger<sup>52</sup> succeeded in demonstrating tubercle bacilli in eggs as early as the eleventh day after artificial infection of healthy hens with avian tubercle bacilli.

Recent work by Fitch and Lubbehusen<sup>53</sup> indicates that probably less than 1 per cent of tuberculous hens lay infected eggs. They divided their tuberculous hens for study into three groups: (1) those that did not lay eggs, (2) those that laid eggs which failed to hatch out and (3) those that laid eggs which produced chickens. These chicks apparently are not usually infected, though Rabinowitsch succeeded in hatching out tuberculous young from infected eggs.

#### SPECIFICITY OF AVIAN TUBERCULIN

On the question of the specificity of avian tuberculin, all shades of opinion may be encountered, from that of Crawford<sup>54</sup> and others, who present evidence of marked specificity, to that of Van Es and others, who think it is a question rather of the strain of bacilli used and of the method of preparation and standardization than of a specific reaction of avian or of mammalian tuberculin. Crawford inoculated one group of guinea-pigs with mammalian, and one with avian, tubercle

51. Wolbach, S. B.: J. M. Research **24**:243, 1911.

52. Raebiger: Beitr. z. Klin. d. Tuberk. **71**:209, 1929.

53. Fitch, C. P., and Lubbehusen, R. E.: J. Am. Vet. M. A. **72**:636, 1928.

54. Crawford, A. B.: Am. Rev. Tuberc. **15**:111, 1927.

bacilli and then subjected them to cross-tests (cutaneous and lethal intraperitoneal reactions) and found marked specificity. Schalk also found that in cattle pastured with droppings from tuberculous chickens there developed a transitory sensitivity only to avian tuberculin. Löwenstein in cases in man found that reactions occurred with much higher dilutions of avian than of human tuberculin. Gasul performed a Pirquet test on 110 tuberculous children who gave positive reactions to human and bovine tuberculin and found that they did not react to avian tuberculin. Plum<sup>23</sup> noticed some specificity in the cattle infected with avian tubercle bacilli. On the other hand, Van Es found that of the cattle into which he had injected avian tubercle bacilli about half reacted to mammalian tuberculin. Dr. Spies had some success in producing an avian tuberculin which reacted in guinea-pigs infected with avian bacilli in higher dilutions than the ordinary mammalian tuberculin.

It appears to me that the only way to settle this point is to isolate from various strains of tubercle bacilli or their filtrates a substance reacting specifically on the skin. In my experience, it is difficult constantly to sensitize guinea-pigs with avian tubercle bacilli. When they give positive reactions the reaction to avian tuberculin is somewhat stronger. On the other hand, it is simple to sensitize guinea-pigs with human bacilli, and they react somewhat more strongly to mammalian tuberculin. In all probability there are present in tuberculin at least two substances reactive on the skin, one specific and one nonspecific, and the proportions of these in a given tuberculin decide its specificity. My own experiences with cases in man are too meager at present to be impressive, yet I have seen a case (sarcoïd) in which the patient reacted as strongly to avian tuberculin as to human, while the majority of the patients who react to human old tuberculin give less reaction with avian tuberculin. It is a point on which more data are needed, and the experiences of the authors quoted suggest it as a fertile field for investigation.

#### ETIOLOGY OF HODGKIN'S DISEASE

Etiologically, Hodgkin's disease has been considered (Stewart and Dobson<sup>55</sup>) as: (1) an atypical form of tuberculosis, (2) a specific infection with a diphtheroid bacillus, (3) a granuloma of unknown etiology, (4) a neoplasm and (5) an initial granuloma which may become neoplastic (Yamasaki<sup>56</sup>). In general it may be said that no one of these possibilities is universally accepted. It is therefore with aroused interest that one reads the report of L'Esperance, who attacked the problem from the point of view of the lesion being an atypical form of tuberculosis due to the avian bacillus. Her experiments are reported

55. Stewart, M. J., and Dobson, J. F.: *Brit. J. Exper. Path.* 5:65, 1924.

56. Yamasaki, M.: *Ztschr. f. Heilk.* 25:269, 1904.

here in some detail. Operative material from 2 fresh cases of histologically characteristic Hodgkin's disease (one from the New York Hospital and the other from the Memorial Hospital, New York) was used. The tissue was macerated in sterile saline solution and within a few hours inoculated intravenously into chickens. The birds, as well as 2 controls, were kept in open air cages in the country. Material from the first case was injected into 2 normal, healthy white Plymouth Rock pullets. One was killed after 5 months, and at autopsy small white foci were found in the liver and spleen which histologically showed peculiar hyalinized necrosis surrounded by large polyhedral cells, giant cells of the myeloid type, many eosinophils and occasional plasma cells. Acid-fast organisms were found in direct smears. The other pullet died after 11 months with similar lesions in the liver and spleen and also involvement of lymphoid nodules in the mesentery and cervical regions. Acid-fast organisms were found in smears and cultures, and on egg medium in 8 days these developed a fine, moist, scant growth of acid-fast organisms and blastomycetes.

Material from the second case was inoculated into 3 white Plymouth Rock pullets. One died in 7 weeks with congenital anomaly of the heart. The liver and spleen showed foci similar to those in the first cases. Material was reinoculated from this hen into 2 other chicks, one of which died in 11 months, showing a mass in the rectal fold and an involvement of the abdominal lymph nodes. Acid-fast bacilli were found. Of the other 2 original birds, one died in 7 months, with many small, opaque areas in the liver, and in the dura mater a nodule that histologically was a granuloma, with little necrosis and many myeloid giant cells. The other was killed in 12 months and showed like foci in the liver. L'Esperance concluded her article with the conservative statement that if one can rule out a spontaneous infection of the birds, it would seem either that material from the lesions of Hodgkin's disease produces a lesion in chickens like tuberculosis or that avian tubercle bacilli are associated with Hodgkin's disease.

More recently, a year after her first papers, L'Esperance reported 2 more cases, 1 of Hodgkin's and 1 of Pel-Epstein's disease (abstracted in foregoing paragraphs) from which avian tubercle bacilli were recovered by inoculation of material into animals. Both of these cases came to autopsy.

In the first case a child, a month following a routine tonsillectomy, began to have a low fever, the temperature ranging to 102 F., accompanied with progressive loss of weight, vomiting and epistaxis. The cervical lymph nodes increased in size for four months; then a node was removed in St. Mary's Hospital, and a histologic diagnosis of Hodgkin's disease was made. A month later the child was admitted to the New York Hospital with a rasping cough and two large swellings in the neck, an enlarged palpable spleen and roentgen evidence of enlarged

mediastinal nodes. There were moderate anemia and leukopenia. At biopsy a diagnosis of Hodgkin's disease was again made. The child died eight months after the onset of the symptoms.

At autopsy 800 cc. of clear fluid was present in the pericardium and a small amount in the pleura, with recent adhesions. Lying in front of the great blood vessels of the neck there was a mass of lymph nodes, the nodes varying from 5 mm. to 3 cm. in diameter. These were traced down on both sides of the aorta to the hilus of the lung, where they projected on the right side into the lung substance and on the left continued downward behind and above the pancreas. The nodes were firm, and the cut surface presented a smooth light brown appearance. The spleen weighed 300 Gm. The surface was slightly irregular, owing to numerous small, greenish nodules. The lungs showed diffuse peribronchial thickenings, with one irregular, firm, ill-defined, grayish-red area in the right apex. There were from ten to twelve large lymph nodes on each side of the neck, with an appearance, on section, similar to that of the mediastinal nodes.

Microscopic examination revealed the histologic picture of typical Hodgkin's disease. Material from the nodes was inoculated into 2 chickens subcutaneously, into 1 rabbit, and into 2 guinea-pigs that had previously been treated with heat-killed tubercle bacilli. One of the chickens was killed after 5½ months and showed early avian tuberculosis; cultures on egg medium yielded a scant, moist growth of acid-fast bacilli. Material from the lesion in the liver reinoculated into chickens produced tuberculosis, which was again carried to a third series of chickens. No mention is made of the second of the originally inoculated chickens. The rabbit inoculated subcutaneously was alive 9 months after the inoculation. Of the 2 inoculated guinea-pigs previously treated by injection of heat-killed mammalian bacilli, 1 died in 50 days with atypical tuberculosis of all the lymph nodes, and cultures on egg medium yielded a moist, pinkish growth of nonacid-fast tiny granules. This culture inoculated into a second "treated" guinea-pig produced extensive tuberculosis of the liver, spleen and lymph nodes. The second originally inoculated "treated" guinea-pig survived 146 days, and at autopsy showed massive generalized tuberculosis. In cultures on egg medium a pure growth of acid-fast bacilli developed with many of the cultural features of avian tubercle bacillus.

It is to be regretted in this instance that no record is given of the second of the originally inoculated chickens, that no original inoculation was made in untreated guinea-pigs, and that all the characteristics of the cultures obtained are not stated, i.e., formation of tuberculin, ability to sensitize infected animals to tuberculin, growth on fluid mediums, acid curve and pathogenicity for guinea-pigs, rabbits and chickens.

One must await repetition of the work before a final judgment can be made, and this is sure to follow shortly on account of the novel method of attack offered in approaching this problem. L'Esperance herself raised the criticism hardest to circumvent, namely, the impossibility, in some cases, of diagnosing tuberculosis in birds, even by the tuberculin skin test, which she neglected to do. One would like to know whether the 2 controls in which lesions developed were in the same cage with the others or whether all the birds were in separate cages, or even whether the 2 different experimental birds were in the same cages. In open cages there is also the possibility of infection

by sparrows or pigeons, whereas Schalk reported persistence of avian tubercle bacilli in soil for 2 years. If one could cultivate acid-fast organisms directly from Hodgkin's nodes, and these had all the characteristics of the bacillus outlined in foregoing paragraphs, one would feel convinced of their presence in the lesions and await only the production of typical Hodgkin's disease in monkeys with such a culture to complete the cycle. To date no account has appeared confirming these observations. Van Es had negative results in 3 cases of Hodgkin's disease; Dr. Aronson of the Henry Phipps Institute in 2 cases failed to isolate the bacilli either by cultivation or by inoculation of chickens. Dr. Schulz<sup>57</sup> inoculated 2 chickens intravenously with a macerated lymph node from a patient at the Children's Hospital, Boston. (Histologic examination showed that the node was not one characteristic of Hodgkin's disease.) One died in 3 months as the result of an obstructed bowel and showed firm, discrete foci in the spleen and very small ones in the liver. Cultures of this organism corresponded to those of the avian type, and intravenous inoculation of a rabbit produced typical tuberculosis in joints, kidneys and lungs. If one excludes this as a spontaneous infection, one may equally well agree that avian tubercle bacilli may occur in the cervical nodes of a child. A second firm node from a case of typical Hodgkin's disease similarly inoculated intravenously into 2 chickens did not produce tuberculous lesions. In this laboratory my associates and I are studying 3 other cases of typical Hodgkin's disease in which the patients do not react to avian tuberculin. Cultures on egg medium have shown no growth, and chickens inoculated and dead within 3 months show no tuberculosis. It appears in conclusion the L'Esperance's work has opened up an interesting field of investigation along the lines of the etiology of Hodgkin's disease. One would also like to see all unusual cases of tuberculosis studied more carefully in order to detect whether infection with avian tubercle bacilli in man in cases other than Hodgkin's disease is prevalent in America. If so, it would necessitate the addition of poultry to the list of inspected meats, since the possibility of infection by eating infected eggs has arisen.

The question has also been raised by Van Es, in view of the large number of swine and cows infected with this organism, whether in the attempts at eradication of tuberculosis a very prevalent source of one form of the tubercle bacillus is being neglected, namely, that in the ordinary barnyard fowl, as the possibility of a transmutation of types is receiving great attention at present.

The difficulties involved in making a diagnosis of infection with avian tubercle bacillus are well illustrated in a case studied by no less an authority than Lydia Rabinowitsch (L. Rabinowitsch-Kempner), reported in detail by Dr. Grunke.

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57. Schulz, R. Z.: Personal communication to the author.



A miller, aged 55, had feverish exanthems and swellings of the limbs resembling erysipelas, with solid infiltrations felt under the skin and new infiltrations forming as existing ones receded. There was no clinical evidence of pulmonary tuberculosis, but there was a positive reaction to tuberculin. Seven months later dry pleurisy developed and death occurred in one month. At autopsy there were a single tuberculous focus in the lung, a discrete tuberculous ulcer at the ileocecal valve and a calcified hilar lymph node. There were an exudative pleurisy and a disease of skin and muscle (tuberculous dermatomyositis), especially of the upper extremities. Large stretches of skin showed reddening and brown pigmentation of the epidermis and subcutaneous edema. Microscopically, there was large caseous tuberculous infiltration around veins and lymphatics. Cultures from the focus in the lung and from the intestinal ulcer, on inoculation into an animal, proved to be typical bovine bacilli. That from the skin, on inoculation into an animal, resembled avian tubercle bacilli, yet Rabinowitsch's conclusion after a two year study of the organism is that in all likelihood it is an atypical bovine organism that during growth in this particular focus took on the character of the avian bacillus.

#### RÉSUMÉ

The avian tubercle bacillus is known to infect certain mammals, notably swine and more rarely cattle. Rabbits, rats and mice may be infected experimentally. There are also in the literature reports of a few cases of infection in man. Cases of tuberculosis of the skin and kidneys, as well as of tuberculosis of septicemic type, due to this organism are on record. Certain authors have reported as also due to this organism certain dyscrasias of the blood (polycythemia rubra and myeloid leukemias). L'Esperance recently raised the interesting point of the possible association of this organism with Hodgkin's disease. One anxiously awaits further detailed studies of her organism, reproduction of the disease with it in monkeys, a larger series of cases and confirmation of her work by other observers. One would like to stress again the precautions that Van Es requires to be taken to minimize the chance of encountering spontaneous tuberculosis in chickens (precautions that are being followed in this laboratory): (1) use of chickens of an age at which the incidence of spontaneous tuberculosis is relatively small—that is, 4 months; (2) selection of birds in which the disease can be excluded; (3) housing of birds off the soil during observation, and (4) a negative reaction to a known potent avian tuberculin in 50 per cent dilution, when a drop of it has been injected intracutaneously into the wattle and the result read in forty-eight and seventy-two hours. A positive reaction consists of a marked swelling of the area.

Should L'Esperance's observations be confirmed, it would open up the possibility of treatment of patients with this known fatal disease by the injection of a vaccine or of an extract of avian tubercle bacilli, and it would rank as one of the outstanding medical contributions of the period.

Finally, it may be said that the avian tubercle bacillus rarely infects man, and that the etiologic rôle of this organism in Hodgkin's disease cannot be accepted as proved.

## News and Notes

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**International Congress of Comparative Pathology.**—The Second International Congress of Comparative Pathology will be held in Paris from October 14 to 18, 1931.

**Division of Medical Sciences of National Research Council Elects Officers.**—The officers of the division of medical sciences of the National Research Council for 1931-1932 are: chairman, William H. Howell; vice chairman, S. Bayne-Jones; executive committee, E. V. Cowdry, S. Bayne-Jones, E. Kennerly Marshall.

## Obituary

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ALDRED SCOTT WARTHIN, M.D.

1866-1931

At the age of 64 and at a time when his joy in creative writing was still in the ascendancy, death came to Aldred Scott Warthin, for thirty-nine years a teacher in the medical school of the University of Michigan and for twenty-eight years professor of pathology and director of the pathological laboratories in that school. Giving freely of his time in attendance at the meetings of the various societies concerned with his special fields of interest, and always taking an active part in discussions, he was well known to most of the internists as well as to the pathologists of two generations.

Dr. Warthin had an interesting and appealing personality which won for him friends wherever he went, notwithstanding a certain degree of reserve. His wide interests in the fields of music, biologic science, gardening, book collecting and medical history enlarged the circle of his acquaintances; and the close contact that he maintained with the clinical aspects of medicine led him into an active part in various organizations not strictly pathologic. Unusually gifted in public address, he was ever generous to the extreme in accepting invitations to speak, and his untiring energy and dynamic and compelling personality will be greatly missed.

Aldred Scott Warthin was born at Greensburg, Ind., on Oct. 21, 1866, the son of Edward Mason Warthin and Eliza Margaret (Weist) Warthin. His early inclination was toward music, and in 1887 he received a teacher's diploma in music at the Cincinnati Conservatory. During the same period he attended Indiana University, from which he obtained the A.B. degree in 1888. At the University of Michigan he earned the A.M. degree in 1890, M.D. in 1891 and Ph.D. in 1893. In 1900, he was married to Katherine Angell, and to them four children were born. From 1891 to 1895, Dr. Warthin served under Dr. George Dock, first as assistant, and later as demonstrator, in internal medicine at the University of Michigan. During this period he spent the summer months of each year in study abroad, giving attention particularly to pathology. He was made demonstrator of pathology in 1896; instructor in 1897; assistant professor in 1899; junior professor in 1902, and professor and director of the pathological laboratories in 1903, holding this rank until his death on May 23, 1931.

Dr. Warthin held membership in numerous societies in the fields of medical biology, internal medicine and pathology, and by many of these organizations he was elected president. Thus he was president of the American Association of Pathologists and Bacteriologists in 1908, of the International Association of Medical Museums from 1910 to 1913, of the American Society for Experimental Pathology in 1924, of the American Association for Cancer Research in 1928, of the Association of American Physicians in the same year and of the American Association of the History of Medicine in 1930-1931. He was a master of the American College of Physicians and had been its first vice-president since 1925.

The hundreds of titles in the list of published works by Dr. Warthin bespeak at the same time his extraordinary energy and industry, and the great breadth of his interests. Only the more important can be mentioned here. During the period in which he was demonstrator in internal medicine, he described in one of his earliest papers the accentuation of the pulmonary second sound in pericarditis and assigned diagnostic significance to it. This has since been known as "Warthin's sign." Tuberculosis of the placenta, the histology and pathology of the hemolymph nodes, the genetic relationship of the leukemias, Hodgkin's disease and lymphosarcoma, the pathology of irradiation, traumatic lipemia, the lesions produced by dichlorethylsulphide (mustard gas) and the constitutional aspects of hyperthyroidism are but a few of the subjects to which he made important contributions. He was best known, however, for his work on the pathology of syphilis. Concerned originally with the lesions of congenital syphilis, Dr. Warthin early recognized the essential unity of the pathology of syphilis as found in various organs and in various stages of the disease. In a series of about forty papers he established a new conception of the pathology of late and of latent syphilis, particularly as it involves the heart, aorta, pancreas, adrenals and testes. Making use of new methods of staining developed in his own laboratory, he was able to support his conception of these disease processes by the demonstration of the causal organism in lesions in which it had never been seen before.

Dr. Warthin's translations and revisions of the tenth and eleventh editions of Ziegler's "General Pathology" and his own "Practical Pathology" and "Autopsy Protocols" have provided useful aids to a long sequence of medical students. He was even more successful in later years as he allowed himself to break away from the concretely practical and to enter the field of the cultural and philosophic aspects of medicine. His early essay on "An American Medical Student" (1903) and many editorials in the *Annals of Clinical Medicine*, of which he had been editor since 1924, have given evidence of his unusual skill in this respect. More recently the three books, "Old Age" (1929), "The



ALDRED SCOTT WARTHIN, M.D.  
1866-1931

Creed of a Biologist" (1930), and "The Physician of the Dance of Death" (1931) have been the product of this reflective outlook on the problems of human life.

As a pathologist, Aldred Scott Warthin was characterized by his adherence to the morphologic approach to the problems of disease, by his ability to recognize the significance of detailed observations and by a far-seeing appreciation of the correlation between structural changes and clinical behavior; as a man, by his untiring energy, broad culture and unswerving loyalty to his friends, to his chosen interests and to the guiding principles of his life.

CARL V. WELLER.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

CHANGES IN SUGAR AND LACTIC ACID CONTENT OF BLOOD CAUSED BY BURNS.  
M. A. SLOCUM and H. D. LIGHTBODY, *Am. J. Physiol.* **96**:35, 1931.

The concentration of sugar and lactic acid in the blood of rabbits after superficial burns was studied both in otherwise intact animals and in animals that had been adrenalectomized. In both there was an increase in blood sugar and in lactic acid, but the increase in the latter was small and insufficient to account for the high value of the sugar. In the animals with intact adrenal glands, the heightened level of the sugar was maintained after twenty-four hours, but lactic acid was reduced below its initial value. Apparently the increased activity of the adrenal glands does not account for the increase in blood sugar through the intermediary of lactic acid, nor for that by decrease in the oxidation rate.

H. E. EGGERS.

THE INNERVATION OF THE RENAL PARENCHYMA. J. KAUFMANN and R. GOTTLIEB, *Am. J. Physiol.* **96**:40, 1931.

A study of the nerve fibers in the renal cortex by means of intravital methylene blue staining and by Bielschowsky's impregnation method showed tubular innervation which seemed to originate in the nerve fibers that accompany the arteries to the tubules. These nonmedullated fibers were found both in the cortex and in the medulla. On the membrana propria they form plexuses, from which come off fine varicose branches which in part end on the membrana propria and in part penetrate the tubules to end between the epithelial cells. Three types of nerve endings were seen: nodular, brushlike and arborized. Not all of the tubules observed showed the presence of nerve filaments, which the writers believe is due to deficiency in method rather than to actual absence. The findings are generally applicable to mammalian kidneys.

H. E. EGGERS.

STUDIES ON DIABETES INSIPIDUS. H. BOURQUIN, *Am. J. Physiol.* **96**:66, 1931.

The effects of injury to the hypothalamus and hypophysis were observed in dogs. In none was there development of dystrophy or of adiposity, and in all in which diabetes insipidus appeared, the injury involved the caudal portion of the hypothalamus. However, no relation was evident between the site of the lesion and the course of the diabetes. In two experiments the infundibulum was separated from the brain stem, and the tuber cinereum was destroyed, and in one the entire hypothalamus was destroyed, with the exception of the caudal tips of the mamillary bodies. In none of these did diabetes insipidus develop, indicating that the disease is not a phenomenon of deficiency. In twenty-six experiments on dogs and in an equal number on rabbits, complete destruction of the hypothalamus and hypophysis did not cause diabetes insipidus; but in twelve of fourteen experiments on dogs, and in twelve of eighteen on rabbits, in which in addition to destruction of the hypophysis and tuber cinereum there was injury to the mamillary bodies, diabetes occurred. Not only is diabetes insipidus not a disease caused by a deficiency, but the mamillary bodies or near-by centers are concerned in its causation, and the polyuria would appear to be caused by a diuretic substance or by irritation of hypothetical centers of control of salt and water metabolism.

H. E. EGGERS.

## FURTHER STUDIES ON THE BASAL METABOLISM OF MAYA INDIANS IN YUCATAN.

G. C. SHATTUCK and F. G. BENEDICT, *Am. J. Physiol.* **96**:518, 1931.

This is a report on a continuation of studies of the basal metabolism of Maya Indians, which were begun in 1927. In both series results were obtained that showed in these Indians a high metabolic rate associated with a low pulse rate and a low blood pressure. In a few cases persons were again observed who had been subjects in the first series, and in these the second determination gave a somewhat lower rate. It is possible that an emotional element contributes to the high rate of these Indians, although their phlegmatic temperament makes this less probable. A third series of determinations is promised.

H. E. EGGERS.

## EFFECTS OF POSTERIOR PITUITARY EXTRACTS ON BASAL METABOLISM.

H. E. HIMWICH and F. W. HAYNES, *Am. J. Physiol.* **96**:640, 1931.

A study was made of the metabolic rate in rats after the injection of pituitary, pitressin and pitocin. With the first, there was a decrease of metabolic rate, due to the pitressin and in spite of the pitocin, with which alone a slight but definite increase occurs. The opposing actions in this respect of pitressin and pitocin presumably account for inconsistencies in previous work along this line.

H. E. EGGERS.

## COMPARATIVE TOXICITY OF THE BLOOD AFTER ARTERIOTOMY, ASPHYXIATION AND INJURIES TO THE BRAIN.

D. I. MACHT, *Am. J. Physiol.* **96**:662, 1931.

Investigated by its effect on plant seedlings, blood from asphyxiated and decerebrated animals of various species was found to be more toxic than fresh arterial blood from the same species. This difference could not be attributed to the anesthetic used nor to a difference in oxygenation, but must be regarded as due to the presence in the blood of toxic substances.

H. E. EGGERS.

## THE DETOXICATING FUNCTION OF THE LIVER WITH SPECIAL REFERENCE TO

STRYCHNINE. J. T. PRIESTLEY, J. MARKOWITZ and F. C. MANN, *Am. J. Physiol.* **96**:696, 1931.

Of the six methods that have been used in studying the detoxicating function of the liver, that in which dehepatized animals and that in which perfusion of the organ are utilized seem to yield the most accurate results. With the use of these methods in dogs the liver was found to have a highly specialized ability to retain rapidly and later to destroy strychnine.

H. E. EGGERS.

## THE RÔLE OF THE LIVER IN THE FORMATION OF LYMPH.

C. MARKOWITZ and F. C. MANN, *Am. J. Physiol.* **96**:709, 1931.

In dogs, a study was made of the flow of lymph through the thoracic duct before and after ligation of the periportal lymphatics. No differences were detected. Investigation of the action of peptone as a lymphagogue in these circumstances in etherized animals likewise revealed no differences, nor did removal of the liver produce any diminution of lymph flow, and the usual lymphagogic effect was observed after the intravenous injection of peptone into a dehepatized dog.

H. E. EGGERS.

## PELLAGRA SECONDARY TO BENIGN AND CARCINOMATOUS LESIONS AND TO DYSFUNCTION OF THE GASTRO-INTESTINAL TRACT.

GEORGE B. EUSTERMAN and PAUL A. O'LEARY, *Arch. Int. Med.* **47**:633, 1931.

Pellagra developed in eight patients with obstructing benign lesions or dysfunction (late, after operation) of the upper part of the digestive tract, in two with obstructing carcinomatous lesions, in one with gastric syphilis and in two with



lesions of the colon (in one carcinomatous; in the other, inflammatory). These cases of secondary pellagra tend to support the theory that dietetic deficiency is the cause of the disease. In the cases reported, the clinical manifestations of the disease were not as marked as those of active, endemic pellagra. Treatment may be ineffectual in cases with mechanical obstruction, or in those with marked impairment of motility, of the upper part of the digestive tract, until the mechanical condition is corrected. Surgical intervention is attended by high mortality. Reports covering the essential clinical features of these cases are submitted.

AUTHORS' SUMMARY.

**HYPERINSULINISM FROM B-CELL ADENOMA OF PANCREAS.** A. D. CARR, R. PARKER, E. GROVE and A. O. FISHER, J. A. M. A. **96**:1363, 1931.

A youth of 19 suffered increasingly from attacks that were similar to the syndrome resulting from an overdose of insulin. They were relieved by the ingestion or administration of dextrose. The increasing severity of the attacks justified exploration and caused the patient and his parents to accept surgical measures. A localized tumor of the pancreas was found and removed. It showed chiefly B-cells. The postoperative recovery was prompt and uneventful, and the clinical cure was complete and was confirmed by studies of the blood sugar.

AUTHORS' SUMMARY.

**COMPENSATORY HYPERTROPHY OF THE SPLEEN.** E. M. MACKEY and W. S. POLLAND, J. Exper. Med. **53**:317, 1931.

In young adult rats the removal of half of the spleen is followed by a compensatory enlargement of the remaining portion of about 50 per cent, while in mature rabbits no such compensatory hypertrophy follows. It is uncertain whether this difference is due to a difference in the function of the spleen in the two species or to the fact that the rats were infected with *Bartonella muris*, while the rabbits were not.

AUTHORS' SUMMARY.

**THE INDUCTION OF LYMPHOCYTOSIS AND LYMPHATIC HYPERPLASIA BY MEANS OF PROTEIN.** B. K. WISEMAN, J. Exper. Med. **53**:499, 1931.

Repeated parenteral injections of protein by various routes in a series of twelve rabbits caused an increase of lymphocytes in the peripheral blood varying from 23 per cent to 139 per cent. It seems probable that the degree of response is conditioned by the type of protein used. At autopsy the lymph nodes and the spleen showed hyperplastic changes. The thymus did not participate in the hyperplasia.

AUTHOR'S SUMMARY.

**EXPERIMENTAL ACUTE GLOMERULITIS.** F. D. W. LUKENS and W. T. LONGCOPE, J. Exper. Med. **53**:511, 1931.

Both focal and diffuse glomerulitis have been produced in rabbits by the injection directly into the left renal artery of suspensions of heat-killed hemolytic streptococci. Similar lesions in the glomeruli could not be obtained in normal rabbits by the injection of suspensions of bismuth oxychloride into the left renal artery. The acute glomerulitis occurred in only about one half of the rabbits employed for the experiments. Glomerulitis was observed much more frequently in rabbits in which an acute localized streptococcal infection had been produced by the intracutaneous injection of living hemolytic streptococci, than in normal rabbits. The occurrence of acute glomerulitis was usually associated with a well marked intradermal reaction to the filtrates of hemolytic streptococci.

AUTHORS' SUMMARY.

EXPERIMENTAL CHRONIC HYPERPARATHYROIDISM LEADING TO OSTITIS FIBROSA.  
A. BODANSKY and H. L. JAFFE, J. Exper. Med. **53**:591, 1931.

On a low intake of calcium hypercalcemia tended to disappear in chronic hyperparathyroidism on a given dose of parathormone (as large as 6 units per kilogram), apparently owing to the reduction of a readily available calcium reserve. An increase of either the intake of calcium or the daily dose of parathormone caused a rise of serum calcium and symptoms of overdosage. Hypocalcemia developed in chronic hyperparathyroidism in young puppies on a diet low in calcium. Tetany occurred at a level of calcium that was higher, and a level of phosphorus that was lower, than in tetania parathyreopriva of young puppies. About 0.1 Gm. of calcium daily was apparently sufficient to maintain the serum calcium at a normal level. The serum phosphorus in chronic hyperparathyroidism in young puppies continued at, or rose above, the high level normal for young animals. Toward the end of long periods of treatment with large doses of parathormone (about 5 units per kilogram) serum phosphorus approached normal levels, and pronounced hypercalcemia was absent, but hypotonia and other symptoms of hyperparathyroidism were present. Early in the treatment and on liberal intakes of calcium, a single dose of parathormone caused more marked relative rise of serum calcium than in normal adult dogs, confirming previous observations. Later in the treatment and on low intakes of calcium, this effect was greatly reduced. Serum phosphorus rose after a single injection of parathormone, even when the effect on the serum calcium was slight or absent. The continued effect of parathormone on serum calcium after prolonged periods of treatment, and the modified response of the serum phosphorus, indicate tolerance due to some compensation, rather than immunity. The lesions of the bones, presenting the essential features of osteitis fibrosa cystica osteoplastica (von Recklinghausen's disease) in varying degrees of severity, depending on the relation of the dose of parathormone to the intake of calcium and to the duration of the treatment, were most prominent on low intakes of calcium, which permitted the use of large doses of parathormone without fatal hypercalcemia and without symptoms of overdosage.

AUTHORS' SUMMARY.

ABSORPTION OF PARTICULATE MATTER BY THE GREAT OMENTUM. A. P. BATCHELDER, M. E. FIELD and C. K. DRINKER, J. Exper. Med. **53**:641, 1931.

When the omentum of the dog is floated in a suspension of insoluble nickel silicate in physiologic solution of sodium chloride under circumstances precluding the possibility of lymphatic drainage, the liver removed after at least one hour contains nickel, which must have been brought to it in particulate form by way of the blood capillaries. The blood capillaries are therefore a pathway for the absorption of solid material, but are not important in this respect.

AUTHORS' SUMMARY.

FIXATION OF BACTERIA AND OF PARTICULATE MATTER AT THE SITE OF INFLAMMATION. V. MENKIN, J. Exper. Med. **53**:647, 1931.

India ink or particles of graphite injected into an area of inflammation fail to disseminate to the tributary lymph nodes. When injected into a normal peritoneal cavity, they rapidly appear in the retrosternal lymph nodes. When injected into an inflamed peritoneal cavity, they are fixed in situ and fail to reach the regional lymph nodes. Particles of graphite injected into the circulating blood enter an inflamed area both as free particles, owing to increased capillary permeability, and also as phagocytosed material within leukocytes. Bacteria (*Bacillus prodigiosus*) injected into inflamed tissue are fixed at the site of inflammation and fail to disseminate to the regional lymph nodes as readily as when injected into normal tissue. Bacteria (*B. prodigiosus*) injected at the periphery of an inflamed area

do not readily penetrate into the site of inflammation. The experiments furnish evidence, in addition to that already provided, that fixation of foreign substances by the inflammatory reaction is primarily due to mechanical obstruction caused by a network of fibrin and by thrombosed lymphatics at the site of inflammation. Bacteria (*B. prodigiosus* and *B. pyocyaneus*) injected intravenously rapidly enter an inflamed area. It is suggested that localization of bacteria in a locus minoris resistentiae may be explained as the result of increased capillary permeability, with subsequent accumulation and fixation of bacteria from the blood stream at the point of injury.

AUTHOR'S SUMMARY.

STUDIES ON TRANSMISSIBLE LYMPHOID LEUCEMIA OF MICE. J. FURTH and M. STRUMIA, *J. Exper. Med.* **53**:715, 1931.

Lymphoid leukemia of the mouse is readily transmitted by intravenous inoculations. The majority of the mice inoculated acquire leukemic lymphadenosis, and a smaller number of them acquire aleukemic lymphadenosis. The data presented favor the view that leukemic and aleukemic lymphadenosis are essentially the same condition. Leukemia produced by transmission is preceded by an aleukemic stage; in this, the lymph nodes and the spleen are uniformly enlarged, and the white blood count and the percentage of lymphocytes are within the normal range, but immature lymphocytes are numerous in the circulating blood. Young, as well as old, mice may acquire leukemia if leukemic material enters their circulation. Studies of transmissible leukemia favor the view that in mammals this is a neoplastic disease. The basic problem of leukemia would seem to be the determination of the factors that bring about a malignant transformation of lymphoid cells.

AUTHORS' SUMMARY.

OBSERVATIONS UPON THE VASCULAR MECHANISM IN ACROCYANOSIS. THOMAS LEWIS and EUGENE M. LANDIS, *Heart* **15**:229, 1930.

The following conclusions are derived mainly from an examination of the hands of a patient with fully developed acrocyanosis. The essential disturbance of the cutaneous circulation is an obstruction in the cutaneous arterioles; the obstruction is not on the venous side. The obstruction is not the result of structural change, but is due to increased tone of the arterioles at the ordinary temperatures of the room. The vessels, though capable of expanding fully, are in a state of relative spasm. This spasm is not due to increased vasomotor tone; its cause is to be sought in the vessels themselves. The arteriolar spasm is the cause of the diminished flow of blood in, and coldness of, the skin. Cyanosis results from the diminished blood flow and also in part from the increased blood content of the skin. The decreased tone of the vessels that give the skin its intense color is due in part to diminished blood flow through them; it is probably brought about in part by the continued low temperature.

AUTHORS' SUMMARY.

MICRO-INJECTION STUDIES OF CAPILLARY BLOOD PRESSURE IN RAYNAUD'S DISEASE. EUGENE M. LANDIS, *Heart* **15**:247, 1930.

Micro-injection studies of capillary pressure in Raynaud's disease indicate that during the period of spasm the pressure at the summit of the capillary loop is between 5 and 8 mm. of mercury when the loop is at the level of the sternum. As the arterial spasm relaxes capillary pressure rises during the hyperemia of recovery to between 32 and 45 mm. of mercury; pulse pressure in the capillaries, absent during spasm, becomes conspicuous, increasing with arteriolar relaxation. A slight swelling of the fingers occurs during recovery and is ascribed to changes in the fluid balance consequent on asphyxia of the capillary wall and on the increased capillary pressure. The slowness with which capillary pressure rises

when venous congestion is artificially imposed during the period of spasm, and the rapidity with which it falls at the release of such congestion, show that this spasm is situated on the arterial and not on the venous side of the capillary network.

AUTHOR'S SUMMARY.

OBSERVATIONS ON DIRECT COMMUNICATIONS BETWEEN ARTERIES AND VEINS IN THE RABBIT'S EAR. R. T. GRANT, *Heart* **15**:281, 1930.

Numerous direct communications between the arteries and veins are constantly present in the rabbit's ear. Their structure is described. The anastomoses can be seen during life and their reactions studied. They react by dilatation to mechanical stimulation, histamine, acetylcholine and cold. Epinephrine constricts them. They can be dilated through the action of the local axon reflex. They are particularly responsive, contracting vigorously and quickly, to sympathetic impulses. An unusually rich distribution of the perivascular sympathetic nervous plexus to the anastomoses is described. The anastomoses serve two functions, (a) local and (b) general: 1. Mainly through their agency, the temperature of the ears is maintained when these are exposed to cold. 2. They are important factors in the regulation of the temperature of the body, aiding the dispersal of heat by allowing an enormous flow of blood through the ears.

AUTHOR'S SUMMARY.

AN ANALYSIS OF THE DATA COLLECTED BY THE STATUS LYMPHATICUS INVESTIGATION COMMITTEE. M. YOUNG and H. M. TURNBULL, *J. Path. & Bact.* **34**:213, 1931.

In the opinion of the committee the facts elicited in the present inquiry are in harmony with those of Hammar (1926 and 1929) and Greenwood and Woods (1927) in affording no evidence that so-called "status thymicolymphaticus" has any existence as a pathologic entity.

AUTHORS' SUMMARY.

MONKEYS BORN IN CAPTIVITY. CHARLES NICOLLE and LOUIS WETTERLÉ, *Arch. Inst. Pasteur de Tunis* **19**:465, 1930.

Two monkeys of the genus *Callitriche* and one of the species *Macacus cynomolgus* were conceived and born in captivity. Conception occurred twice during convalescence from a serious infection on the part of one parent. The advantages of raising monkeys in studies of genetics and of the transmission of infections are evident.

M. S. MARSHALL.

CHANGES IN THE BLOOD AND RENAL FUNCTION AFTER REMOVAL OF THE CELIAC AND SUPERIOR MESENTERIC GANGLIONS AND THE LEFT ADRENAL GLAND. P. PANNELLA, *Riv. di path. sper.* **6**:132, 1930.

After removal of the celiac and superior mesenteric ganglions and the left adrenal gland, the red blood cells are unchanged, but the white cells are increased in number about 200 per cent. A retention of chloride was produced in the blood, and a disturbance of renal function could be shown through the xanthoprotein test.

EMMERICH HAAM.

CHANGES IN THE LUNG AFTER REMOVAL OF THE CERVICAL PART OF THE SYMPATHETIC NERVE. D. VALLONE, *Riv. di pat. sper.* **6**:278, 1930.

The removal of the cervical part of the sympathetic produces hyperemia in the lung by vasoparalysis. This hyperemia is bilateral, but more marked in the

lung of the side on which the operation was performed. A vital staining of the lung with trypan-blue shows a scarcity of the blue stained granules in the lung of the side operated on, indicating trophoneurotic disturbances of this organ.

EMMERICH HAAM.

HEMORRHAGIC DIATHESSES. M. B. SCHMIDT, Verhandl. d. deutsch. path. Gesellsch. **25:10**, 1930.

In the main, the author concludes from an anatomic and experimental study that many of the conditions grouped under "hemorrhagic diathesis" may be explained by changes in the vascular walls, especially those of the venules. He urges that in the study of these conditions more attention be paid to the blood vessels. In his consideration of the various hemorrhagic diatheses he reaches the following conclusions:

*Hemophilia*.—No changes are found in the walls of the blood vessels. The possibility of chemical changes in the blood must be seriously considered. A similar picture is found in deep icterus and phosphorus poisoning, in which there is a disturbance of the formation of thrombokinase from injury to the liver. A clumping together of the thrombocytes in a slow circulation, without the formation of fibrin, causes an endothelial reaction that results in vascular rupture (Dietrich). This may explain the hemorrhages in hemophilia. One must not confuse this condition with pseudohemophilia, in which there is an absence of fibrinogen. This fact and the absence of a family history should exclude hemophilia vera.

*Thrombocytopenia*.—Thrombocytopenia may occur alone or secondary to infections or to diseases of the blood. That this condition is associated with thrombopenia does not mean that all hemorrhagic diatheses may be so explained. The cause of the thrombopenia, whether it is a decreased formation of platelets in the bone marrow (Frank) or an increased destruction in the spleen (Kaznelson), has not yet been determined. In a case of sudden generalized hemorrhage, the author found a mild infectious state of the blood vessels, especially of the small veins, with endothelial proliferation and perivascular round cell infiltration, but no bacteria. The bone marrow was normal, with no decrease in number or change of the megakaryocytes. From these observations the author concluded that there is no constant relationship between thrombopenia and hemorrhagic diathesis, and that the vascular changes are probably the most important factor.

*Scurvy*.—The deficiency of vitamins predisposes to infection by organisms already present in the skin, mouth, etc. In the skin the hemorrhages are about hair follicles because of infection there. The deep hemorrhages are also explained on an infectious basis, as the small veins show proliferation of the endothelium with perivascular round cell and leukocytic infiltration. Experimental scurvy in guinea-pigs shows similar histologic changes. Superimposed infection causes an increase in the hemorrhagic diathesis.

*Panmyelophthisis (Aplastic Anemia)*.—Here, too, changes in the small veins are found where hemorrhages occur. In experimental benzene poisoning of rabbits hemorrhages were noted before the platelet count diminished, indicating that there is a toxic change in the wall of the blood vessels, and that the decrease in the number of platelets is not essential.

*Schönlein-Henoch Purpura*.—In this condition changes are found in the smaller blood vessels, similar to those described. The rôle of thrombi, as stressed by Silbermann, could not be verified. As it is difficult to reproduce a similar condition experimentally, little can be added.

*Infectious Type*.—Emboli of bacteria, as in meningococcic meningitis and bacterial endocarditis, and toxic changes of the endothelium, as in hemorrhagic small-pox, are the underlying factors in infectious conditions.

*Uremia*.—In uremia, a cytolsin is produced that dissolves the capillary endothelium as snake venom does.

SOL ROY ROSENTHAL.

## Pathologic Anatomy

SHADOWS PRODUCED BY LEAD IN THE X-RAY PICTURES OF THE GROWING SKELETON. EDWARDS A. PARK, DEBORAH JACKSON and LASLO KAJDI, Am. J. Dis. Child. **41**:485, 1931.

Four cases of lead poisoning occurring in infants are reported. Three of the infants had definite histories of having ingested paint; the other possessed an unreliable history in this regard. Two of the cases were fatal, and in these autopsies were performed. In all the cases roentgen examination revealed clouding of the bone in the area of greatest growth, namely, the metaphysis. The changes were similar to those produced by ingestion of phosphorus, arsenic and some of the heavy metals. It is suggested that clouding appeared only in the areas of most rapid growth because the lead combined with the cells there or altered them. Microscopic sections through these areas of density revealed an increase in the number of trabeculae and more marked compression of them. In the two fatal cases the band shadows (metaphysis) were narrow and intense; this probably indicates massive doses of lead over a short period of time. The roentgen shadows that are produced may be of value in the diagnosis of lead poisoning in children.

P. H. GUINAND.

A CASE OF AMYOTONIA WITH HISTOLOGIC OBSERVATIONS. C. R. TUTHILL and M. G. LEVY, Am. J. Dis. Child. **41**:591, 1931.

The case of amyotonia described occurred in a boy who was fully developed at birth and who was the first born child of young and healthy parents. Attention was drawn to a weakness of the lower limbs when the child was 10 days of age. At 1 month the child showed pronounced paralysis of the lower limbs, which spread gradually to the muscles of the upper limbs, of the neck and of deglutition and to the accessory muscles of respiration. Scoliosis and ankle drop also developed. The tendon reflexes were lost, but at no time was sensation involved. In the sixth week the child began to lose weight. He died of aspiration pneumonia when he was 10 weeks of age. Gross and microscopic examination of the skeletal muscles showed the changes typical of Oppenheim's disease or of Werdnig-Hoffman's muscular atrophy. This was true also of histologic examination of the nervous system, in which were found chromatolysis of the ganglion cells, neuronophagia and loss of cells in the anterior horns of the spinal cord, in the hypoglossal nuclei and in the thalami. The anterior roots of the cord and the fibers of the hypoglossal nerve were atrophied. In the only muscle examined, the tongue, the motor plate endings were not present. This case does not offer any points of differentiation, either clinically or pathologically, between Oppenheim's disease and the Werdnig-Hoffman type of muscular atrophy.

AUTHORS' SUMMARY.

CORRELATION OF THE ROENTGENOLOGIC PICTURE WITH THE ANATOMIC CHANGES IN CONGENITAL OSSEOUS SYPHILIS. STAFFORD McLEAN, Am. J. Dis. Child. **41**:607, 1931.

A complete clinical, roentgenologic and pathologic study was made of sixteen infants who died at the age of 13 months or less. In this group all types of syphilis of the bones were represented. The clinical findings strongly indicated the diagnosis of syphilis, which in every case was supported by either biopsy or autopsy. Thirteen of the cases presented positive serologic results: in the other three cases the blood was not examined. In every case the diagnosis of osseous syphilis was substantiated by roentgen examination.

P. H. GUINAND.

A TOOTH IN THE PLEURAL CAVITY. I. DAVIDSOHN, *Am. J. M. Sc.* **181**:494, 1931.

As it is assumed that the deciduous teeth are shed not later than the age of 12 or 13 years, about from nineteen to twenty years had elapsed in the case reported since the tooth, being accidentally inhaled into the left bronchus, began its migration into the pleural cavity. It is possible that the point of entrance was not at the place where it was found at autopsy. The adhesions along the lateral aspect of the lung may point to a perforation higher up with a subsequent inflammation due to irritation. The tooth itself sank to the lowest point, and there it was found at autopsy. The long time that intervened between the inhalation of the tooth and the patient's death may explain the absence of any evidence of the destructive path that the tooth had traveled. The striking feature of this case is the absence of any detectable pathologic changes in the lung, with the exception of the pleural adhesions. Also of interest is the long time during which the tooth was in this unusual location without occasioning symptoms.

AUTHOR'S SUMMARY.

ABSORPTION FROM THE PLEURAL CAVITY OF DOGS. GEORGE M. HIGGINS and WILLIS S. LEMON, *Am. J. M. Sc.* **181**:697, 1931.

The polymorphonuclear leukocyte, so actively phagocytic in early exudates, disintegrated without further transformation. The large number of mononuclear cells arose from the locally produced wandering cell, the blood monocyte and perhaps the lymphocyte. The majority of the mononuclear cells continued as spherical ameboid cells, whereas many others produced connective tissue fibers and resembled fibroblasts. The cells resembling fibroblasts which appeared in the fully organized exudative cellular masses may be derived from both clasmatoocytes and lymphocytes, from locally produced fibroblasts and from the mesothelial cells of the parietal pleura.

AUTHORS' SUMMARY.

HEMORRHAGIC PANCREATITIS. V. J. DARDINSKI, *Am. J. Path.* **7**:169, 1931.

In two cases of hemorrhagic disease of the pancreas impaction of gallstones at the ampulla of Vater could not be considered an etiologic factor. In one case the common bile duct and the pancreatic duct each had a separate opening on the tip of the papilla. In the other case the outlet of the pancreatic duct was 5 mm. distal to that of the common bile duct. In neither case was there any possibility that the obstruction at the outlet produced a continuous channel from the common bile duct into the pancreatic duct.

AUTHOR'S SUMMARY.

OBSTRUCTION OF THE AQUEDUCT OF SYLVIVS. SAMUEL T. ORTON, *Bull. Neurol. Inst., New York* **1**:72, 1931.

One of Orton's two cases of occlusion of the sylvian aqueduct was due to a pronounced granular ependymitis; the other, to a small astrocytoma. The complaints in the first case were unsteady gait, rapid gain in weight, excessive appetite, especially for sweets, and polyuria. The symptoms developed within three years. The patient was short in stature, obese and dull in appearance and showed a papilledema and basal metabolism of —10. Roentgenograms and ventriculograms showed only increased intracranial pressure and dilated ventricles. The patient died eight days after the ventriculography.

Macroscopic and microscopic observation showed an extensive internal hydrocephalus caused by a pericanalicular gliosis and granular ependymitis of the sylvian aqueduct; the fourth ventricle showed changes similar to those around the aqueduct. The left ventricular wall was ruptured with formation of a cyst, compression of the vermis, collapse of the roof of the fourth ventricle and a consequent secondary obstruction to the ventricular outflow of fluid.

In the second case, a boy, aged 9, had his forehead and one leg injured in an automobile accident two years before admission. Three months before admission, bed wetting and pollakiuria (at night) set in, also increase in weight, occasional headache and visual disturbances. Examination revealed bilateral papilledema, a suggestion of binasal hemianopia, a rather large sella turcica and no air shadows in the ventricles (after injection of air by lumbar puncture). Two operations performed for a pituitary lesion revealed no tumor. The patient died about three weeks after the first operation. A diagnosis of a probable tumor of the third ventricle had been made. Necropsy revealed hydrocephalus and occlusion of the sylvian aqueduct by an astrocytoma fibrillare of exceedingly small size, though Orton admits that a differentiation between a small fibrillary astrocytoma and a proliferative gliosis is often rather difficult; that is, pathognomonic morphologic differences are absent. The most consistent difference between the neoplastic and the other types of growth Orton sees in the evidence of nuclear activity, as shown by variation in size, form and staining reaction. In the tumor, the size of the nuclei and their staining qualities are much more variable; often the nuclei are of double volume; most of them are vesicular; they are hardly ever pyknotic, and budding and twin nuclei are rather common. Additional factors are the extent of the tumor, complete obliteration of the parenchyma and its replacement by the tumor and a greater wealth in cells. Of the conclusions, the most interesting is that enlargement of the head from infancy may indicate a reduced factor of safety in ventricular drainage and may serve as a symptom of importance in differentiating structure of the aqueduct from tumors of the posterior fossa and from suprasellar growths.

GEORGE B. HASSIN.

#### TUMOR OF THE POSTERIOR CRANIAL FOSSA CAUSING VISUAL HALLUCINATIONS.

EDWIN M. DEERY, *Bull. Neurol. Inst., New York* 1:97, 1931.

Tumors of the occipital and frequently also of the temporal lobes have been known to cause visual hallucinations by direct or indirect involvement of the optic pathways. Deery reports two cases in which optic hallucinations occurred with cerebellar tumors, in which thus the possibility of a lesion of the optic nerve could with safety be excluded. In the first case, papilledema was associated with signs of involvement of the fifth, seventh and eighth nerves; yet a supratentorial tumor of the temporal lobe was diagnosed because of the predominance of hallucinations and of signs of involvement of the pyramidal tract on the left side (exaggerated reflexes and some spasticity). Necropsy revealed a tumor of the left cerebellar pontile angle, which the author defines as an acoustic neuroma.

In the second case the hallucinations were both olfactory and visual, with signs of a cerebellar tumor, revealed mainly by roentgenogramis. An operation showed a dermoid cyst of the middle cerebellar lobe. A definite anatomophysiologic explanation is not given by the author.

GEORGE B. HASSIN.

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### Microbiology and Parasitology

AGRANULOCYTOSIS. WILLIAM DAMESHEK and MAURICE INGALL, *Am. J. M. Sc.* 181:502, 1931.

Nine cases diagnosed as agranulocytosis are reported: Four of the cases were typical of the "angina agranulocytica" described by Schultz; the other five were atypical in one or another feature. Two of the cases occurred in infants. In two of the cases recovery took place. In one case injections of arsphenamine constituted in all probability an etiologic factor. The diagnosis of the disease, the blood pictures and various clinical features are discussed. The phase of recovery, with its marked monocytosis and histiocytosis, was studied in cases



7 and 8. It is felt that agranulocytosis is a symptom complex, dependent primarily on an abnormal reaction of the bone marrow to severe sepsis. Gradations can be seen between the typical case with angina and cases of sepsis with an atypical leukopenia.

AUTHORS' SUMMARY.

A CASE OF ENDEMIC TYPHUS IN VIRGINIA. H. PINKERTON and K. F. MAXCY, *Am. J. Path.* 7:95, 1931.

Study of a fatal case of endemic typhus fever in Virginia has established the complete pathologic identity of the disease with European typhus, the characteristic lesions of the brain were fully as numerous as in the average case of European typhus fever, and many of these lesions were unusually acute. Involvement of the tunica vaginalis and scrotum was no more marked than in European typhus fever. *Rickettsia prowazeki* was easily and clearly demonstrated in the endothelial cells of the vascular lesions in the brain, but were not found in macrophages or neuroglial cells. A technic is described for the demonstration of rickettsiae in formaldehyde fixed brain. The demonstration of *Rickettsia prowazeki* in this material is to be added to the already overwhelming evidence in favor of their etiologic relationship to typhus fever.

AUTHORS' SUMMARY.

SUSCEPTIBILITY OF THE GOPHER TO TUBERCULOSIS. W. H. FELDMAN, *Am. J. Path.* 7:139, 1931.

Gophers were inoculated intravenously, subcutaneously and intraperitoneally with human, bovine and avian strains of the tubercle bacillus. The most pronounced infection was noted in animals inoculated intravenously. Infection with all three strains was possible, but that with the human and bovine strains was the most pronounced.

EDNA DELVES.

PATHOLOGICAL REACTION OF DOGS TO AVIAN TUBERCULOSIS. W. H. FELDMAN, *Am. J. Path.* 7:147, 1931.

Dogs intracerebrally inoculated showed tuberculous lesions in the brain and liver in all instances. In one case the spleen was involved. Lesions were never found in the lungs. Intravenous injection gave, at times, a nonprogressive lesion limited to the liver. The lesions produced were circumscribed or diffuse accumulations of monocytic cells. Most lesions were progressive; necrosis was not common, and giant cells were absent. Attempts to produce infections through other portals of entry indicated that the dog possesses a constitutional resistance to avian tuberculosis.

EDNA DELVES.

RESISTANCE OF PROTOZOA TO CROTALUS AND COBRA VENOMS. C. H. PHILPOTT, *Biol. Bull.* 60:64, 1931.

In an attempt to determine whether the effect of venoms on various types of protoplasm is general, a study was made of the relative resistance of fourteen species of protozoa to the lethal action of crotalic and cobra venoms. It was found, so far as this group of protozoa is concerned, that the poisonous effect of these venoms is general. There was found to exist among the species, however, much variation in the degree of resistance to these agents. Some species had relatively high resistance to one venom and low to the other while, in other species, the reverse relationship existed.

CHARLES H. PHILPOTT.

REDUCTION OF RESISTANCE TO TYPHUS VIRUS BY DIET DEFICIENCY. H. ZINSSER, M. RUIZ CASTANEDA and C. V. SEASTONE, JR., J. Exper. Med. **53**:333, 1931.

The experiments demonstrate that guinea-pigs and rats subjected to diets deficient in vitamins to a point at which symptoms appear, and then inoculated with typhus virus, exhibit clinical pictures that indicate a far more severe infection than that observed in normal animals after inoculation. There is also a wider distribution of *Rickettsiae* and a concentration of organisms which, in pleural and peritoneal exudates, amounts almost to cultural proportions. Important from our point of view is the fact that these experiments furnished a step toward the accomplishment of our purpose, which was to obtain amounts and concentrations of *Rickettsiae* suitable for immunologic studies until such time as tissue culture may have developed to a practically useful stage. The experiments are of immediate importance in that they furnish us a method for improving the technic of active immunization reported on in the preceding paper, no. 5. From the epidemiologic point of view these experiments at least suggest an explanation of one of the important factors that enter into the historical association of high mortality from typhus with war and famine.

AUTHORS' SUMMARY.

FURTHER OBSERVATIONS ON THE SURVIVAL OF VACCINE VIRUS SEPARATED FROM LIVING HOST CELLS BY COLLODION MEMBRANES. R. S. MUCKENFUSS, J. Exper. Med. **53**:377, 1931.

The survival of vaccine virus separated from a suspension of fresh minced rabbit kidney by a collodion membrane was not complete in these experiments, and passage in series was not successful. The degree of survival seemed somewhat greater if dead cells, killed by repeated freezing and thawing, were added to the virus during incubation, although the tissue was able to increase the intensity of the intradermal reactions. Extracts of dead cells did not increase the degree of survival, as determined by the intensity of the intradermal reaction. No significant or constant increase in the intensity of the intradermal reactions resulted from the addition of cysteine hydrochloride to the virus in the dialyzing apparatus.

AUTHOR'S SUMMARY.

ETIOLOGY OF COMMON COLD. P. H. LONG, J. A. DOULL, J. M. BOURN and E. McCOMB, J. Exper. Med. **53**:447, 1931.

Experimental infections of the upper respiratory tract similar to "common colds" were transmitted singly and in series through two and four passages in nine of fifteen persons, by intranasal inoculations with bacteria-free filtrates of nasopharyngeal washings obtained from persons ill with natural "colds." These observations conform with those reported by previous workers and lend further support to the view that the incitant of the "common cold" is a filtrable virus.

AUTHORS' SUMMARY.

VARIATION AND TYPE SPECIFICITY IN THE SPECIES *HEMOPHILUS INFLUENZAE*. M. PITTMAN, J. Exper. Med. **53**:471, 1931.

Strains of influenza bacilli are of two kinds, which have been called S and R. The S strains are distinguished by the appearance of their colonies, which have a smooth surface, are of large size, show opaqueness and take on iridescence in oblique transmitted light, by the fact that the individual bacteria are capsulated, and by the fact that they produce a soluble specific substance which is present in culture filtrates and in washings of the bacteria. R strains form colonies that are rough and irregular in outline, are less opaque than S colonies, are of smaller size and are not iridescent; the individual bacterium possesses no capsule, and these strains produce no soluble specific substance. The S strains are also more

pathogenic for animals than the R strains. By means of cross-precipitation reactions it has been possible to divide the fifteen S strains studied into two distinct immunologic types. The same specific types are shown by means of agglutination reactions carried out at a temperature of 37 C. Spontaneous conversion of S into R strains occurs in artificial culture mediums with great readiness. This may be delayed by certain cultural procedures, and may be hastened by growth in mediums containing type-specific antiserum. Artificial conversion of R into S strains has been observed, but the changes are carried out only with great difficulty.

AUTHOR'S SUMMARY.

EPIDEMIOLOGY OF PNEUMOCOCCUS INFECTION. L. T. WEBSTER and T. P. HUGHES, J. Exper. Med. **53**:535, 1931.

Pneumococci were found in the nasal passages and throats of 80 per cent of 105 adults and children studied. Ninety-seven per cent of 500 strains studied were serologically specific. Pneumococci of types I and II were found under conditions suggesting their lack of capacity to spread. Types III and XIII seemed to spread from person to person. Individuals were found to be pneumococcus-free or transiently, periodically or chronically carriers. The incidence of pneumococci in all persons studied underwent a seasonal variation paralleling that of coryza and sore throats in the same person.

EDNA DELVES.

METABOLISM OF S AND R FORMS OF PNEUMOCOCCUS. P. FINKLE, J. Exper. Med. **53**:661, 1931.

In the present paper are given the results of studies on the respiratory and glycolytic metabolism of pneumococcus types I, II and III, and of the R forms derived from these. The S and R forms are compared as to metabolism, and the relationship between changes in virulence, changes in chemical constitution and changes in metabolism is discussed.

A BACTERIOLOGICAL STUDY OF "COLDS" ON AN ISOLATED TROPICAL ISLAND. D. F. MILAM and W. G. SMILLIE, J. Exper. Med. **53**:733, 1931.

Studies in the Virgin Islands, Labrador and Alabama suggest that colds are incited by some specific agent with which investigators are not yet familiar. These studies suggest also that the secondary and more severe symptoms associated with colds may be due to certain aerobic flora commonly found in the nasopharynx. Types of pneumococci that are virulent (in white mice) and true Pfeiffer's bacilli requiring both V and X substance (and forming indol?) seem to be of particular importance in these secondary infections. The studies indicate that the specific agent that initiates colds is infectious, and is spread by direct contact, with an incubation period of from one to three days. There is strong evidence that environmental factors, particularly reduction in atmospheric temperature, have some influence on the incidence of colds.

AUTHORS' SUMMARY.

THE EFFECT OF SECONDARY INFECTIONS ON EXPERIMENTAL TRACHOMA. P. K. OLITSKY, R. E. KNUTTI and J. R. TYLER, J. Exper. Med. **53**:753, 1931.

By introducing secondary infections in monkeys already showing characteristic granular conjunctivitis following inoculation of human trachomatous tissues or cultures of *Bacterium granulosis*, it is possible to bring about a condition the clinical appearance of which closely resembles that of florid human trachoma. Secondary infection appears to be important in the pathogenesis of the experimental disease, since by it a reaction that is mainly follicular can be converted into a still more severe and destructive hyperemic, granulopapillary type. In this respect an analogue may be found to trachoma in man.

AUTHORS' SUMMARY.

UNDULANT FEVER IN AZERBAIDJAN. P. ZDRODOWSKI, H. BRENN and B. VOSKRESSENSKI, Ann. Inst. Pasteur **45**:768, 1930.

In a lengthy study of undulant fever over the past eight years, the authors considered clinical aspects, treatment, pathologic anatomy, allergy, infection of animals and the organisms of the genus *Brucella*. Fifteen persons who contracted the disease in the laboratory were observed; in six of these the symptoms were almost negligible, although one had a positive blood culture and a titer of agglutination of 1:10,000. In from 80 to 85 per cent of the cases, blood cultures showed organisms, but the titers of the serum agglutinin varied. Latency of seven months was noted. Enduring nervous symptoms may exist, as well as orchitis, adenitis, mastitis, subcutaneous abscess, icterus et al. Various treatments were uncertain as to results. All organisms were virulent for guinea-pigs; virulence was demonstrated after eight years' isolation. Infection was noted in parenteral, oral and intradermal inoculations and in inoculation of the conjunctival sac. In guinea-pigs symptoms were usually absent, but might be acute; cachexia and alopecia, keratitis, abscesses, paralysis, with involvement of joints, and orchitis might be noted. A unique opportunity for study was afforded in a natural epizootic among 400 guinea-pigs. A typical mobilization of cells was noted in the lymph nodes, spleen and bone marrow; it was less extensive elsewhere. Histologically the lesions resembled those of pseudotuberculosis. No superinfection seemed possible, and culture filtrates proved innocuous. A series of studies of the organisms indicated the presence of normal smooth S types and rough R types, with some intermediates. The paratypes of *B. melitensis* and *B. abortus* were found to be R in type, and the R types were avirulent or weakly virulent. There were noted numerous serologic varieties. In the opinion of the authors, any classification of the organisms involved is arbitrary.

M. S. MARSHALL.

CHICKENPOX-HERPES ZOSTER VIRUS. ARNOLD NETTER and ACHILLE URBAIN, Ann. Inst. Pasteur **46**:17, 1931.

Using complement-fixation and studying a number of cases of chickenpox and of herpes zoster-like exanthems from various angles, the authors suggest that there is a close relationship between the viruses of these diseases.

M. S. MARSHALL.

EPIDEMIOLOGY OF POLIOMYELITIS. C. LEVADITI, E. SCHMUTZ and L. WILLEMIN, Ann. Inst. Pasteur **46**:80, 1931.

In this article are presented the results of a thorough epidemiologic study of an epidemic during the months from June to September, 1930, in the vicinity of Strasbourg, involving 405 persons (60.3 per hundred thousand), of whom 39 died.

M. S. MARSHALL.

POLIOMYELITIS. C. LEVADITI and L. WILLEMIN, Ann. Inst. Pasteur **46**:233, 1931.

Following an epidemiologic report in a preceding number of this journal, an experimental study is given. From the spinal cords of five patients who died in the early stages, four successful inoculations were made in monkeys. Difficulty was encountered in serial transfer. The cerebral cortex in these cases gave negative results. In a case in which the spinal cord induced symptoms, the sciatic nerve gave negative results. Inguinal lymph nodes produced an abortive form of the disease, and tonsils removed during the evolution of poliomyelitis in a child induced definite symptoms in a monkey. Twice, in eight attempts, positive results were demonstrated with nasopharyngeal filtrates. Fecal filtrates killed animals inoculated with them, suggesting a toxin, before a reasonable period of incubation. Per os or on scarified nasal mucosa neither unfiltered nasopharyngeal secretion

nor feces conferred the disease. Water and milk, potentially naturally containing the virus, gave negative results. The virus survived at least two days in several species of mosquitoes tested, but direct transmission by this route failed. Flies did not appear to retain the active virus. Transmission by human contact is the favored hypothesis.

M. S. MARSHALL.

PNEUMONIC PLAGUE IN TUNIS. PAUL DURAND and ERNEST CONSEIL, Arch. Inst. Pasteur de Tunis **19**:245, 1930.

An outbreak of pneumonic plague occurred in Tunis during the latter part of December, 1929, and the early part of January, 1930. There were sixty-five cases, all of which, except four, were fatal. This paper presents the results of clinical, pathologic and bacteriologic studies. A preceding paper by these authors considered epidemiologic observations. The period of incubation varied from two to three days. The sudden onset of symptoms, ending with cyanosis and death, is discussed. Death followed in less than four days in half of twenty-two cases studied. One case exceeded eight days. At autopsy the bodies were frequently cyanotic, with purple lips, and the skin marked with bloody effusions—"the 'black death' of the middle ages." Rigidity was marked. There was inflammation of the pleura. The lungs showed areas of hepatization and violet zones of congestion and edema; hepatization was rarely red as in frank pneumonia and appeared microscopically as a mass of cells, white cells and bacilli being numerous. The tissue was firm, did not recede under pressure and sank in water. The heart was usually dilated, and some fluid was contained in the pericardium. The liver and the spleen were slightly congested; the kidneys appeared normal. The intestines were usually congested, and the peritoneal cavity contained some fluid. Blood cultures, after four or five days' incubation, were usually positive in life or at autopsy. After the first day of the disease, sputum yielded excellent cultures. The lungs, and to a less extent the liver and the kidneys, yielded cultures at autopsy. Patients given vaccine by spraying the respiratory tract yielded cultures in the sputum late, if at all; puncture of the lung demonstrated their presence. No mixed infections were noted.

M. S. MARSHALL.

BRUCELLA INFECTIONS IN TUNIS AND IN MALTA. I. F. HUDDLESON, Arch. Inst. Pasteur de Tunis **19**:391, 1930.

The organisms of *Brucella* were most frequently demonstrated in the blood during a rise in fever in the patient. All strains isolated from cases in man in Tunis and in Malta were, by the author's methods, of the caprine or *B. melitensis* type, i. e., neither bovine nor porcine varieties. Cases were found to be more acute and to last longer.

M. S. MARSHALL.

TUBERCLE BACILLEMIA IN VARIOUS DISEASES. E. LOEWENSTEIN, München. med. Wchnschr. **78**:261, 1931.

Loewenstein reports that with his cultural technic the tubercle bacillus has been isolated repeatedly from the blood of patients.

EDWIN F. HIRSCH.

TRANSPLACENTAL TUBERCULOUS INFECTION. M. P. ISABOLINSKI and W. J. GITOWITSCH, Zentralbl. f. Bakt. (Abt. 1) **116**:491, 1930.

Tubercle bacilli passed through the placentas of tuberculous guinea-pigs and infected the fetuses, miliary tubercles being found in the lungs, liver and spleen, as well as tubercle bacilli and Much granules.

PAUL R. CANNON.

THE FILTRABILITY OF TUBERCLE BACILLI AND OF OTHER BACTERIA. FRANZ LUCKSCH, *Zentralbl. f. Bakt. (Abt. 1)* **117**:1, 1930.

Lucksch finds that granular developmental forms of the tubercle bacilli may be found in filtrates (Berkefeld and Chamberlin) in the sputum of tuberculous patients. The granules are not peculiar to tubercle bacilli, however, as they may also be found in filtrates from actinomycetes and many other micro-organisms. He believes that they develop within the animal body and cause lesions. They are probably forms of the bacteria modified by adverse environmental conditions and should not be considered as filtrable viruses.

PAUL R. CANNON.

A LABORATORY INFECTION WITH SCARLET FEVER. F. VON BORMANN, *Zentralbl. f. Bakt. (Abt. 1)* **117**:460, 1930.

A woman, aged 20, while washing filters used in filtering cultures of streptococci from the throat washings of patients with scarlet fever, acquired an infection of the left hand. From twelve to fourteen days later typical scarlet fever developed, although the patient had not been in contact with any cases. Hemolytic streptococci, culturally identical, were obtained from the wound and from the nasopharynx. The erythema produced by the filtrate from these organisms was neutralized by scarlet fever antiserum.

PAUL R. CANNON.

THE CAUSES OF THE ILLNESS IN INFANTS FOLLOWING VACCINATION WITH CALMETTE-GUÉRIN BACILLI IN LÜBECK. B. LANGE, *Ztschr. f. Tuberk.* **59**:1, 1930.

This is the final report from the Robert Koch Institute on the work that was done to decide what caused the death of numerous infants in Lübeck following vaccination with BCG. The conclusions are as follows: The fatalities were caused directly by the oral administration of the vaccine prepared in Deycke's laboratory. The bacilli isolated from three infants dead of tuberculosis were pathogenic and caused progressive tuberculosis in guinea-pigs. Bacilli isolated from another infant who had died of intercurrent disease were not pathogenic for guinea-pigs. It is more than likely that the vaccines used consisted of a mixture of true BCG bacilli and a fully pathogenic strain of human bacilli. It must be assumed that the mixture was different at various times during the period of administration. There is not the slightest evidence that the BCG had increased in virulence.

MAX PINNER.

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## Immunology

COMPLEMENT FIXATION IN YELLOW FEVER IN MONKEY AND IN MAN. GORDON ERNEST DAVIS. *Am. J. Hyg.* **13**:79, 1931.

Serums from monkeys that have recovered from undoubted attacks of yellow fever invariably fix complement in the presence of virus-containing serums used as antigens. Serums from normal monkeys or from monkeys that have survived experimentation without infection as evidenced by death from yellow fever following the inoculation of a virus known to be active fail to fix complement in the presence of the same antigens. Serums from monkeys that have received successive doses of active virus, but that have shown no thermal reaction as evidence of yellow fever, fix complement in the same degree as serums from monkeys convalescent from yellow fever. Serums from monkeys that have received successive doses of inactive virus fail to fix complement. Serums from monkeys that have received inactive virus ("vaccine") followed by active virus may or may not

fix complement. Serums from monkeys that have been passively immunized with serum from convalescent monkeys and then inoculated with active virus do not fix complement unless there has been an apparent lowering of the immunity, as indicated by a thermal reaction to the virus. Serums from monkeys that have received convalescent human serum and active virus may or may not fix complement. Serums drawn from monkeys during the febrile period of the disease act as potent antigens. The antibody content of serums from monkeys convalescent from yellow fever is demonstrable in a few days following the termination of the febrile period and may remain fairly constant over a period of several months. Serums of monkeys that have survived yellow fever, which have given complement fixation, but which after some weeks give a weaker reaction, or in which complement-fixing substances can no longer be demonstrated, may be brought to the original titer or to one much higher by the inoculation of fresh or inactivated virus-containing serum (55 C. for fifteen minutes). Complement fixation in yellow fever in monkeys does not depend on concomitant bacterial infection. Passive sensitization of both guinea-pigs and rabbits is additional evidence of a true antigen-antibody relationship in complement fixation in yellow fever. The rapid rise in the titer of the serum of a protected monkey following the inoculation of macerated infected mosquitoes is evidence of a biologic specificity. The biologic specificity of the complement fixation in yellow fever will probably remain undetermined until an antigen has been obtained that is less complex than blood serum or tissue extracts. Complement fixation, as used at present, can be of no assistance in determining areas of endemic yellow fever.

AUTHOR'S SUMMARY.

HUMAN TRICHINELLA SPIRALIS INFECTIONS AND THE BACHMAN PRECIPITIN TEST. GEORGE W. HUNTER, Am. J. Hyg. **13**:311, 1931.

Six cases presenting histories and clinical courses suggestive of trichinosis are discussed. In three that offered a common source of infection the Bachman test was positive, although biopsy on specimens taken from the gastrocnemius muscle gave negative results. The factors responsible for the eosinophilia are probably the factors reacting in the test positive for precipitin. They are most evident in the third or fourth week of the infection. The hope for an early diagnosis of these cases rests in an intracutaneous test. The test for precipitin is an advance over the biopsy, but needs refinement. The possible value of the test for precipitin in diagnosing cases in livestock is mentioned.

P. H. GUINAND.

ACTIVE IMMUNIZATION AGAINST TYPHUS FEVER WITH FORMALINIZED VIRUS. H. ZINSSER and M. RUIZ CASTANEDA, J. Exper. Med. **53**:325, 1931.

We have adduced evidence that guinea-pigs can be completely or partially protected by three injections of typhus tunica material in which there are moderate numbers of *Rickettsiae*, treated for from twenty-four to forty-eight hours with a 0.2 per cent solution of formaldehyde. We believe that the immunization is due to the presence of *Rickettsiae*, since in our preceding experiments we satisfied ourselves that these organisms are the true etiologic factors of the disease. For the reasons stated, we believe that the formaldehyde vaccine does not contain living, but attenuated, organisms, and that the immunizing effect is the result of treatment with formaldehyde-killed *Rickettsiae*. This point, however, we admit, is not absolutely determined. These experiments, with the results obtained in the concentration of *Rickettsiae* in material by the dietary method of reducing resistance, as described in the paper that follows, furnish a hopeful method and a reasonable theoretical basis for a procedure of active immunization against this disease in human beings.

AUTHORS' SUMMARY.

## NON-SPECIFIC LOCAL CUTANEOUS IMMUNITY TO STAPHYLOCOCCUS AUREUS.

J. A. TOOMEY and S. O. FREEDLANDER, J. Exper. Med. **53**:363, 1931.

Many substances besides the filtrates of broth cultures of *Staphylococcus* (Besredka) can be utilized as topical applications to protect guinea-pigs from the effects of massive doses of staphylococci given subcutaneously (plain broth, 10 per cent peptone, 1 per cent peptone, Liebig's meat extract, mustard plaster and normal horse serum). Where such protection occurs, no matter what the stimulus is, the local cutaneous reaction microscopically is the same as that previously described for broth compresses. Many topical applications of such substances as saline solution, water, plain compresses, etc., may confer slight protection on an animal. Specific filtrates (Besredka) confer no protection on the animal if applied at the time of inoculation or thereafter. The local protection described in our experiments is nonspecific.

AUTHORS' SUMMARY.

IMMUNIZATION WITH MIXTURES OF POLIOMYELITIS VIRUS AND ALUMINUM HYDROXIDE. C. P. RHODES, J. Exper. Med. **53**:399, 1931.

Experiments are presented that indicate that the virus of poliomyelitis can be inactivated by a certain preparation of aluminum hydroxide. This effect is seen at neutrality and at a hydrogen ion concentration of  $p_{H}$  5.5, but not at that of  $p_{H}$  8.8. Monkeys treated by repeated subcutaneous injections of the virus so inactivated evince immunity, as shown by resistance to intranasal instillation and intracerebral inoculation, as well as by the neutralizing power of their serums. The treatments that give rise to the immunity produce no symptoms of disease.

AUTHOR'S SUMMARY.

## ACTIVE IMMUNIZATION AGAINST MEXICAN TYPHUS FEVER WITH DEAD VIRUS.

H. ZINSSER and M. RUIZ CASTANEDA, J. Exper. Med. **53**:493, 1931.

Guinea-pigs can be immunized against the virus of Mexican typhus by intraperitoneal injections of formaldehydized rickettsia-containing material, provided sufficient amounts of the organisms are used. Our results in this respect are analogous to those of Spencer and Parker with phenolized virus of Rocky Mountain spotted fever. Suspensions of *Rickettsiae* appear to possess considerable toxicity. We do not wish to be misunderstood as implying that the results in guinea-pigs offer anything more than a demonstration of the principle of active immunization with killed *Rickettsiae*. The application to man will have to be worked out, and preliminary to this we are now attempting to apply the methods to monkeys.

AUTHORS' SUMMARY.

IMMUNOLOGICAL RELATIONSHIPS AMONG THE PNEUMOCOCCI. J. Y. SUGG and J. M. NEILL, J. Exper. Med. **53**:527, 1931.

This paper reports interreactions of anaphylaxis and precipitation between antigens and antisera derived from *Pneumococcus*, type II, and from one variety of yeast. That the reactions occurred only with type II and not with types I and III is proof that the pneumococcal antigen responsible for the anaphylaxis of the animals sensitized to yeast was the type-specific carbohydrate (S). Pronounced differences in respect to reactivity with the pneumococcal antigen were found between antiyeast sera that were of equal potency in respect to reactivity with the yeast. This fact emphasizes the desirability of the use of separate antisera from a large number of animals in the study of microbial interrelationships.

AUTHORS' SUMMARY.



POTENT ANTIPOLIOMYELITIC HORSE SERUM CONCENTRATE. E. R. WEYER, W. H. PARK and E. J. BENZHAF, J. Exper. Med. **53**:553, 1931.

The horse, apparently itself not susceptible to poliomyelitis, can be stimulated in certain cases, but not all, to the production of virucidal antibodies. The virucidal potency of such immune serum can be raised to a point comparable with that of human convalescent serum, and when concentrated and refined, it exhibits a fourfold increase in potency. Such concentrates have proved effective in the prevention of paralysis in inoculated monkeys when given intraspinaly before the onset of paralysis. Treatment has been found to be more effective when the therapeutic serums have been given by the spinal route. Serum from "normal" adult donors has proved effective in neutralizing virus, but its potency is approximately one-half that of serum from convalescent persons.

AUTHORS' SUMMARY.

SPECIFIC AND NONSPECIFIC POLYSACCHARIDES OF TYPE IV PNEUMOCOCCUS. M. HEIDELBERGER and F. E. KENDALL, J. Exper. Med. **53**:625, 1931.

Three nitrogen-containing polysaccharides have been isolated from autolyzed cultures of *Pneumococcus*, type IV: a carbohydrate specific for the type, differing markedly from those of types I, II and III, and representing a type of substance hitherto not observed among specific polysaccharides; a chemically similar carbohydrate without specific function, and the "C" substance, or species-specific polysaccharide of Tillett, Goebel and Avery. The chemical differences between the specific polysaccharides of *Pneumococcus* are discussed, and the relationship of the new examples to chitin is pointed out, as well as the bearing of this relationship on the unsettled controversy as to whether or not chitin occurs in bacteria. The data of Tillett, Goebel and Avery on the "C" substance have been extended.

AUTHORS' SUMMARY.

CONGENITAL PROTEIN HYPERSENSITIVENESS IN TWO GENERATIONS. B. RATNER and H. L. GRUEHL, J. Exper. Med. **53**:677, 1931.

Hypersensitivity actively induced in utero is shown to persist for a longer period than passive sensitization. The degree of hypersensitivity, its duration and its transmissibility appear to be influenced by the time elapsing between the original injection of the sensitizing substance into the parent and parturition. A pregnant guinea-pig receiving a parenteral injection of antigen from two to four days prior to parturition transmits a state of hypersensitivity to two succeeding generations. The sensitization of the first generation is due to the passage of antigen. The sensitization of the second generation is due to the passage of antibodies formed in the first generation. This prevents any further transfer of the hypersensitive state. Though hypersensitivity occurs in two successive generations, the phenomenon is congenital and not hereditary. We believe that this phenomenon demonstrated in the guinea-pig is fundamentally related to the problem of congenital sensitization of the human being.

AUTHOR'S SUMMARY.

ANTIBODIES IN THE URINE AFTER INJECTION OF ANTITOXIC GLOBULIN. J. M. NEILL, E. L. GASPARI and J. Y. SUGG, J. Immunol. **20**:187, 1931.

Tests for diphtherial antibodies were made on the urine of a child with a mistaken diagnosis of diphtheria who had received intramuscular injections of a large amount of the usual antitoxic horse globulin. The presence of the antibody to the toxin was proved both by the capacity of the urine to neutralize the toxin in vitro and by in vivo neutralization of the toxin injected subcutaneously into guinea-pigs several hours subsequent to intravenous injection of the urine. Therapeutic solutions of the diphtherial antitoxin contain not only the antibody to the toxin, but also an antibody of the antibacterial sort, recognizable by

agglutination of a degraded strain of diphtheria bacilli; a certain amount of these antibacterial antibodies was also contained in the urine of the child who had received the therapeutic solution. No serum protein was detected in the urine either by the usual chemical tests or by anaphylactic tests. The failure to detect the globulin in the urine was not considered evidence of a separation of the antitoxin from the globulin, because both the chemical and the anaphylactic tests proved unable to detect the globulin in dilutions of therapeutic antitoxic globulin equivalent in concentration of antitoxin to that in the child's urine.

## AUTHORS' SUMMARY.

THE ANAPHYLACTOGENIC ACTION OF THE PROTEIN FROM FILTRATES OF ACID-FAST BACTERIA. J. H. LEWIS and F. B. SEIBERT, *J. Immunol.* **20**:201, 1931.

The proteins isolated from filtrates of acid-fast bacterial cultures on a synthetic medium are actively anaphylactogenic. By use of the anaphylactic reaction it can be shown that a definite antigenic relationship exists between the proteins in the three types of tubercle bacilli and none between the tubercle bacilli and the timothy bacillus. Guinea-pigs infected with human tubercle bacilli are actively sensitized to the protein of tuberculin. This sensitization appears from four to six weeks after infection. Precipitating serums from guinea-pigs immunized with the protein of tuberculin can passively sensitize normal guinea-pigs. Precipitating serums from tuberculous guinea-pigs are very feeble in their ability to confer passive sensitization, despite the high titers of their precipitins.

## AUTHORS' SUMMARY.

ANAPHYLACTIC AND TUBERCULIN TYPES OF HYPERSENSITIVENESS. L. DIENES, *J. Immunol.* **20**:221, 1931.

Tuberculous guinea-pigs sensitized with egg-white and also with various other antigens often die after intraperitoneal injection of these substances in a protracted shock that is similar in many respects to the shock following injection of tuberculin. It is of special significance that a strong hemorrhagic reaction develops around the tuberculous lesion. This hemorrhagic reaction is not the result of the special sensitiveness of the lesion; it might be present in any inflammatory area without regard to the origin of the inflammation. The shock from egg-white differs in two points from that from tuberculin. The symptoms often develop early after the injection, and in the early phases of the reaction and in the slight reactions a drop in temperature is usually present. These differences are probably connected with the presence of the usual anaphylactic sensitiveness after treatment with egg-white. When the latter is associated with sensitiveness to tuberculin, the shock from tuberculin often presents the same temperature curve and early onset of symptoms as the shock from egg-white. The capacity to react with severe, protracted shock develops later after the treatment than either the tuberculin type of cutaneous sensitiveness or the anaphylaxis. We observed it only in actively sensitized guinea-pigs, and our attempts to transfer it passively remained unsuccessful. The different manifestations of the hypersensitive condition develop in a large measure independently from each other. The independence of the severe, protracted shock from the anaphylactic shock is shown also by the observation that the desensitization to the acute anaphylactic shock does not interfere with the development of the severe, protracted shock. The severe, protracted shock from egg-white and the shock from tuberculin are probably analogous processes, but without a more thorough understanding of the physiologic mechanism of the reaction this conclusion must be regarded as only provisory.

## AUTHOR'S SUMMARY.

GROUP-SPECIFIC HUMAN HEMAGGLUTININS. I. DAVIDSOHN, *J. Immunol.* **20**:239, 1931.

It was possible in a relatively small percentage of cases to remove partly or completely the group agglutinogens from the human red corpuscles by the mechanical procedure of repeated washing and shaking with physiologic solution of sodium chloride, thus confirming the results of Schuetz and Wohlsch and of Hallauer. It is possible to produce group-specific anti-A and anti-B immune serums by adsorbing the heterologous agglutinins in rabbit immune serums with the corpuscles A or B, respectively. By using unwashed human red blood corpuscles a relatively high percentage of group-specific agglutinating serums is obtained.

AUTHOR'S SUMMARY.

TRANSMISSION TO THE THIRD GENERATION OF ANTITOXIN. J. Y. SUGG, L. V. RICHARDSON and J. M. NEILL, *J. Immunol.* **20**:255, 1931.

A guinea-pig of the second generation transmitted to its own offspring (third generation) small amounts of antitoxin originally derived by active immunization of the first generation. Previous reports of failure of transmission were probably due to a quantitative inadequacy of the immunity originally transmitted to the females of the second generation to persist to the time of birth of the third generation.

AUTHORS' SUMMARY.

THE INFLUENCE OF HYPERSENSITIVENESS TO HORSE SERUM ON THE DURATION OF LOCAL PASSIVE ANTITOXIC IMMUNITY IN MAN. S. B. HOOKER and E. M. FOLLENSBY, *J. Immunol.* **20**:269, 1931.

The duration of a local passive immunity produced by injecting scarlatinal antitoxin into the skin was compared in human subjects who were separable into three groups according to their endermic reactivity to horse serum. Those who responded negatively or with a reaction of the delayed type usually retained an effective portion of the test dose of antitoxin for more than twenty-four hours; those who reacted immediately disposed of antitoxin so much more rapidly that in less than four hours an equally effective portion was no longer demonstrable. That this accelerated disposal of antitoxin is specifically dependent on the allergic state seems highly probable; certain evidences that were not directly conformable to this hypothesis have been discussed.

AUTHORS' SUMMARY.

EXTIRPATION OF THE ANTIGENIC DEPOT AND ANTIBODY PRODUCTION. EDWARD F. ROBERTS, *J. Immunol.* **20**:291, 1931.

A single injection of a minute amount of antigen suffices to stimulate the production of antibodies. Extirpation of the site of antigenic deposition as soon as ten seconds after inoculation serves to diminish slightly the extent of the production of antibodies. The amount of antigenic stimulation afforded has an appreciable, but not proportional, effect on the extent of the production of antibodies. Antigen is immediately transported from its site of parenteral deposition to other parts of the body. The stimulus to the production of antibodies is the antigen itself, and this necessitates contact with the antibody-producing tissues. The initial stimulus is far more effective than are succeeding stimuli in determining the rate and the extent of the production of antibodies. The transmission of the stimulus is neither a function of the nervous system nor a reflex-like process, but simply the transportation of the antigen itself by way of the veins supplying the area inoculated.

AUTHOR'S SUMMARY.

THE ULTRAMICROSCOPIC PRECIPITATION REACTION IN SYPHILIS. A. M. MALLOY and R. L. KAHN, *J. Infect. Dis.* **48**:243, 1931.

Studies on the nature of the ultramicroscopic reaction between antigenic suspension (Kahn) and syphilitic and nonsyphilitic serums led to the following results: The aggregates in the antigenic suspension and the precipitates in syphilitic serum have the same appearance under the ultramicroscope. The molecular complexes of antigenic suspension seem to form the structural units in building up the precipitates in serum. Both syphilitic and nonsyphilitic serums possess the property of precipitation when mixed with antigenic suspension, syphilitic serum in a marked degree and nonsyphilitic serum in a slight degree. When a mixture of serum and antigenic suspension is shaken for several minutes, the reaction is brought practically to completion and is little affected by incubation. Without shaking, incubation aids precipitation. Sensitized antigenic suspension and syphilitic serum give results similar to those with standard antigen. Nonsyphilitic serum reacts more with sensitized than with standard suspension. Syphilitic rabbit serums give the same ultramicroscopic picture as syphilitic human serum. The same holds true as to nonsyphilitic rabbit serums, except that after prolonged incubation these show a greater tendency toward precipitation than do nonsyphilitic human serums.

AUTHOR'S SUMMARY.

THE TOXIN-ANTITOXIN UNION. A. V. STOESSERT, *J. Infect. Dis.* **48**:255, 1931.

All experiments with the watery extract of the mushroom, *Amanita phalloides*, as toxin and its immune rabbit serum as antitoxin have given results that are compatible with the theory that the union with neutralization of the antigen, toxin and the antibody, antitoxin, is a colloidal adsorption phenomenon. This colloidal union is slower, but firmer, at temperatures much below that of the room, such as 4 C., and rapid and more dissociable at a higher temperature, such as 37 C. When a firm union has been formed, after a sufficient length of time has elapsed, the toxin and the antitoxin cannot be dissociated by simple dilution (with isotonic saline solution), regardless of the temperature at which they were permitted to interact. The effect of simple dilution on this adsorption union shows that in getting quantitative relationships between the toxin and the antitoxin, the concentration of each is probably as important as the absolute amount of each present. In combining with each other, toxin and antitoxin behave as colloids, but the specificity of this process of neutralization is not explained by present knowledge concerning colloids.

AUTHOR'S SUMMARY.

NEUTRALIZATION OF SKIN REACTION BY ANTIMENINGOCOCCUS SERUMS. G. SHWARTZMAN, *J. Infect. Dis.* **48**:339, 1931.

The potency of antimeningococcus serums was measured by the phenomenon of local skin reactivity. The majority of serums at present applied therapeutically are poor in neutralizing antibodies. A prolonged period of immunization with toxic filtrates and live cultures proved necessary for the development of potent serums. Group and "variant" specificity of meningococcal toxic filtrates was demonstrated.

FROM AUTHOR'S SUMMARY.

BRUCELLA ABORTUS. R. GWATKIN, *J. Infect. Dis.* **48**:381, 1931.

Injections of killed suspensions, filtrates (heated and unheated), ground bacterial extracts and suspensions, and formaldehydized suspensions gave no evidence in guinea-pigs of protection to subsequent infections with living *Brucella abortus*.

EDNA DELVES.

IMMUNITY IN THE LUNGS OF RABBITS FOLLOWING IMMUNIZATION WITH PNEUMOCOCCI. G. W. STUPPY, P. R. CANNON and I. S. FALK, J. Prev. Med. 5:97, 1931.

The mechanism of the active immunity produced by vaccination with pneumococci in these experiments may be somewhat as follows: The disposal of the pneumococcal bodies by the phagocytes of the lung during the course of the immunization stimulates the phagocytic tissues to increased functional activity, as well as to the production of increased numbers of macrophages. Antibodies are also probably produced locally, as well as generally, during this process. The later intrabronchial insufflation of living pneumococci is followed by a more active response of the pulmonary tissues because of the previous "sensitization" by vaccination. The pneumococci are localized and destroyed within the pulmonary tissues because of the increased activity and larger numbers of macrophages available, as compared with those in the normal lung. As a part of this process, polymorphonuclears may have an important rôle in the early stages. Furthermore, agglomerating and opsonizing immune bodies may facilitate the more rapid removal of the pneumococci. The reaction is predominately cellular, although obviously not independent of humoral agencies.

FROM AUTHORS' SUMMARY.

IMMUNIZATION WITH STAPHYLOCOCCUS FILTRATES. G. M. DACK, E. O. JORDON and O. WOOLPERT, J. Prev. Med. 5:151, 1931.

Four human subjects fed with gradually increasing doses of staphylococcal filtrates, reaching amounts of from 25 to 32 cc., appeared to acquire some tolerance to the poison of the particular strain used in the immunization. Heterologous filtrates (10 cc.) taken at the conclusion of the "immunizing" treatment produced no symptoms in one subject. Two other volunteers, however, when fed respectively 2 and 10 cc. of the filtrate of a heterologous strain, showed definite symptoms, which in the person receiving the larger dose were violent. A fifth subject was extremely sensitive to small doses of filtrate and did not acquire any tolerance. Rabbits given intravenous injections of gradually increasing doses of staphylococcal filtrates acquired tolerance both toward the staphylococcal poison of the strain used in immunization and toward that of a heterologous strain. Serum from four normal persons, from a patient with active chronic osteomyelitis and from one with an old healed lesion of osteomyelitis, when injected along with staphylococcal filtrate, did not protect rabbits against the staphylococcal poison. Serum from "immunized" men and rabbits did not protect monkeys or rabbits when it was mixed with potent filtrates and injected intravenously, nor did such serum protect a human volunteer who swallowed it mixed with a potent filtrate.

AUTHORS' SUMMARY.

TUBERCULIN SENSITIVITY IN RATS. HARRY SCHUTZE and S. S. ZILVA, Brit. J. Exper. Path. 11:489, 1930.

Rats fed a diet deficient in fat-soluble vitamins A and D become more sensitive to the lethal effects of big doses of tuberculin after infection with *Bacillus tuberculosis*, but a similar sensitivity is displayed by the tuberculous rat toward the toxic effects of a suspension of killed organisms of the genus *Salmonella*. The response is therefore not to be regarded as a specific one.

AUTHORS' SUMMARY.

THE CONCENTRATION OF THE PROTECTIVE SUBSTANCE IN ANTIPOLIOMYELITIS SERUM. W. T. J. MORGAN and R. W. FAIRBROTHER, Brit. J. Exper. Path. 11:512, 1930.

From the experimental results obtained with the serum from a horse immunized with living poliomyelitic virus, it would appear that the protein precipitated at a relatively low concentration of ammonium sulphate is uniformly much more potent in antiviral action per unit weight of protein than that precipitated at higher concentrations of this salt; and, in conformity with this result, the protein thrown

out of solution by dialysis of the immune serum and the serum protein that is insoluble in saturated solution of sodium chloride has been shown to possess potent antiviral properties. The evidence indicates that in this serum there is a general distribution of the antibody throughout the serum proteins, since carefully fractionated preparations of the pseudoglobulin and albumin possess definite, although slight, virus-neutralizing properties.

AUTHORS' SUMMARY.

THE ABSORPTION OF DIPHTHERIA ANTITOXIN. A. T. GLENNY, A. G. HAMP and M. LLEWELLYN-JONES, Brit. J. Exper. Path. **12**:21, 1931.

There is a considerable difference in the total amount of antitoxin absorbed into the blood stream by different guinea-pigs given subcutaneous injections of the same amounts of serum. No conclusions can be drawn from experiments on a limited number of animals. There is no significant difference in the amount of antitoxin absorbed as between that given diluted and that given undiluted. The rate of absorption of antitoxin depends on the amount of protein injected compared with the weight of the animal. The amount of antitoxin absorbed in six hours may be reduced by the addition of a great excess of protein, to about 25 per cent of that absorbed without such addition. The influence on the rate of absorption of the amount of protein injected is greatest when the latter is large in relation to the weight of the animal. The average concentrated therapeutic serum is absorbed at least as rapidly as unconcentrated serum containing the same number of units when small doses are injected and more rapidly when large doses are given.

AUTHORS' SUMMARY.

SEROLOGICAL VARIETIES OF TYPHUS FEVER. A. FELIX and M. RHODES, J. Hyg. **31**:225, 1931.

Fletcher and Lesslar's observations on two serologic types of tropical typhus have been fully confirmed. The antigenic relationship between the indologenous *Bacillus proteus* X 19 and the nonindologenous Kingsbury strain is of the same order as that obtaining between the X 19 and X 2 types of *B. proteus* X. The Kingsbury strain is an antigenic variant derived from the original X 19 culture and represents another serologic type of *B. proteus* X. The symbol X K is suggested for this type. Serums from persons with classic European typhus and from persons with the endemic typhus of the United States of America and of Australia have been tested for the occurrence of main and group O agglutinins for the known types of *B. proteus* X. H agglutination as a source of error in the diagnosis of typhus is illustrated by some examples. Serums from persons with the tsutsugamushi of Sumatra and Japan react with type X K like those from Malaysians with this disease described by Fletcher and his co-workers. The latter reaction is of the order of group O agglutination. It is suggested that antigenically the virus of tsutsugamushi corresponds to a serologic type of *B. proteus* X that is yet unknown. The data published on the reactions of the serums of persons with Rocky Mountain spotted fever and of persons with the "fièvre exanthématique" of Marseilles are analyzed. It is suggested that these two diseases represent further serologic varieties of typhus. The significance hitherto attached to negative results in agglutination tests with *B. proteus* X and to those in cross-immunity tests obtained in some cases of typhus-like disease requires revision in the light of recent observations.

AUTHORS' SUMMARY.

THE INTRACUTANEOUS METHOD OF TESTING DIPHTHERIA TOXIN AND ANTITOXIN. A. T. GLENNY and M. LLEWELLYN-JONES, J. Path. & Bact. **34**:143, 1931.

The intracutaneous method of titrating diphtherial toxin and antitoxin is more convenient, rapid and accurate than the subcutaneous method. By the intracutaneous method it is possible to determine the presence of as little as  $\frac{1}{50,000}$  unit of antitoxin in 0.1 cc. of serum.

AUTHORS' SUMMARY.

PASSIVE IMMUNITY WITH PNEUMOCOCCUS III. L. COTONI and N. CHAMBRIN, Ann. Inst. Pasteur **45**:706, 1930.

Various antigenic preparations were injected into both rabbits and horses over varying periods of time. Complete protocols are tabulated for the rabbits used. The antigens were prepared by drying the sediments from centrifugated cultures at 35 C., or by using alcohol-ether dried material. The injections were made by subcutaneous, intramuscular and intravenous routes. The animals withstood the injections well. The serums showed antihemolytic power, not specific for type, but agglutinins and precipitins were rarely noted. The protective properties were tested on mice by injecting 0.1 cc. and 0.2 cc. of serum subcutaneously, followed the next day by from 100 to 10,000 minimum lethal doses of *pneumococcus*, type III. Of forty-eight rabbits, variously inoculated, twenty-nine produced protective serum. The best results were obtained, both in rabbits and in horses, with small doses of dried antigen.

M. S. MARSHALL.

INFLUENCE OF CALMETTE-GUÉRIN BACILLI ON ANIMALS INFECTED WITH TUBERCULOSIS. I. LEVITAN, D. LOKHOFF and V. KOSMODEMIANSKI, Ann. Inst. Pasteur **45**:740, 1930.

No modifications of a general tuberculous infection were noted, as compared with controls, in guinea-pigs and rabbits given injections of BCG (from 2 to 19 doses), though the survival was somewhat greater. Little difference in the pathologic anatomy was apparent. The lesions, particularly those in the lymph nodes and in the liver, seemed to develop a more fibrous character in vaccinated animals surviving for a relatively long period. There was a slight systemic response to the injections of vaccine in tuberculous animals.

AUTHORS' CONCLUSIONS.

IMMUNITY IN EXPERIMENTAL PLAGUE. A. COMPTON, Ann. Inst. Pasteur **45**:754, 1930.

The therapeutic efficiency of serum and of bacteriophage was studied in mice experimentally infected with plague. Experiments in prophylaxis were also carried out, in which use was made of Haffkine's vaccine and bacteriophage, some of which had been treated with formaldehyde and was lytically inactive. Serum in large doses had apparent therapeutic value, but the bacteriophage was not effective. However, the bacteriophage, both the normal filtrate and that treated with formaldehyde, had some value as a prophylactic agent. Animals treated with one or two doses of bacteriophage showed in some instances a state of hypersusceptibility, but with three injections protection was good. Immunity thus established seemed to be antibacterial and antitoxic, and to be greater than that attained with Haffkine's vaccine.

M. S. MARSHALL.

NERVOUS SYSTEM AND REFLEXES IN IMMUNITY. S. METALNIKOV, Ann. Inst. Pasteur **46**:137, 1931.

Certain defensive reactions occur in response to rubbing or to warming of the skin of previously immunized animals. On this basis, a hypothesis that immunity is a vital phenomenon, like digestion or respiration, is developed. To the usual response to bacteria, considered an adaptation, is added a reaction having its seat in the nervous system, considered a defensive reaction of general significance. Experimental observations involved the injection of heated bacteria of various species, followed by a study, under stimulation, of leukocytosis, agglutinins and degree of protection.

M. S. MARSHALL.

PURIFICATION AND CONCENTRATION OF DIPHTHERIAL TOXIN. S. SCHMIDT, A. HANSEN and K. A. KJAER, *Ann. Inst. Pasteur* **46**:202, 1931.

Toxin and toxoid (anatoxin) were purified by means of aluminum hydroxide, with a coincident concentration of nearly a hundred times. Material so prepared presented good flocculation with antitoxin and had good immunizing potency in guinea-pigs. Trials in progress (Madsen) indicate that in man the immunizing power is high and the reactions are notably lower than with the standard product.

M. S. MARSHALL.

PULVERIZED PLAGUE VACCINE VIA THE RESPIRATORY TRACT. CHARLES NICOLLE, PAUL DURAND and ERNEST CONSEIL, *Arch. Inst. Pasteur de Tunis* **19**:267, 1930.

Vaccination by spraying was attempted in the outbreak of pneumonic plague in Tunis (from December, 1929, to January, 1930). Other methods were also attempted. Of 866 isolated contacts, 503 were vaccinated subcutaneously and 363 by way of the respiratory tract (50 of the latter also receiving subcutaneous injections). In the first group there were 6 cases and 5 deaths; in the second group there were 3 cases and 1 recovery. Those in the second group were considered to have been in closer contact with cases than those in the first group. In a subsequent paper on the same subject, dealing with rural cases (Georges Villain, p. 277), it is concluded that this method of vaccination resulted in the rapid cure of colds or banal coryzas, and that conditions favorable to the development of the plague bacillus might be suppressed.

M. S. MARSHALL.

SERUM PROPHYLAXIS IN MEASLES. CHARLES ANDERSON and F. GÉRARD, *Arch. Inst. Pasteur de Tunis* **19**:435, 1930.

Following the principles implied in the idea of local immunization, persons who had come in contact with patients with measles but who had not previously had the disease were treated thrice daily by a drop of convalescent serum in each eye. Although only thirty patients are reported on thus far, all of those treated in this way escaped the disease, whereas measles developed in other contacts within the usual period.

M. S. MARSHALL.

THE RIECKENBERG REACTION. S. INOUE, *Zentralbl. f. Bakt. (Abt. 1)* **117**:80, 1930.

The Rieckenberg reaction, which manifests itself in the adhesion of blood platelets to spirochetes in the presence of their immune serum, was studied in detail. With *Spirochaeta duttoni* it may be obtained at the temperature of the room, reaching its maximum in from twenty to thirty minutes, is only obtained with living, motile spirochetes, and can be secured with bacteria and granules of ink, as well as with platelets, these all serving merely as indicators of increased adhesiveness of the spirochetes as influenced by the immune body. The latter is in the serum, as well as in the plasma, is destroyed by heating at 72 C. for thirty minutes, is strongly specific, is formed by the injection of killed spirochetes and can be demonstrated in the fetuses of an immune mother. By this reaction, in combination with agglutination, etc., *S. icteroides* (Noguchi) was found to be identical with *S. icterohaemorrhagiae*. In the serums of five patients ill of Weil's disease, the Rieckenberg reaction occurred with *S. icterohaemorrhagiae* seven days after the beginning of the illness and again five months later.

PAUL R. CANNON.



## Tumors

SALTING OUT OF THE AGENT OF CHICKEN TUMOR WITH THE GLOBULIN OF FILTRATES. M. R. LEWIS and W. MENDELSON, *Am. J. Hyg.* **12**:686, 1930.

It is evident that in tumor extract the virus itself may be of a globulin nature or it may become attached to the precipitated particles of globulin. If the latter, the virus is not fixed to the globulin but can be released in the tissue of the chicken and there bring about the growth of a tumor. Isolation of the virus will depend largely on the elucidation of the problem as to which type of union exists between the virus and the globulin.

AUTHORS' SUMMARY.

INTRAMEDULLARY TUMORS OF THE SPINAL CORD. JAMES W. KERNOHAN, HENRY W. WOLTMAN and ALFRED W. ADSON, *Arch. Neurol. & Psychiat.* **25**:679, 1931.

The tendency to classify the tumors of the central nervous systems, especially the glioma group, according to the type of embryonic cells they contain and their evolution was mainly directed toward the brain tissues. Tumors of the spinal cord were much less studied along these lines, for they are rarer. Kernohan, Woltman and Adson had the unusual opportunity to study such tumors and classify them according to the latest standards. Of a total of ninety-one intramedullary tumors, it was possible to study microscopically fifty-one cases. The conclusion the authors arrived at is that the tumors of the spinal cord in no way differ from such tumors in the brain; that is, the types of tumors found in the spinal cord are the same as those present in the brain. The exception is spongioblastoma multiforme which is rare in the spinal cord.

The tumors found were: astroblastoma, medulloblastoma, hemangioblastoma and ganglioneuroma, and one tumor classified as fibroma, one as lipoma and one as tuberculoma. In five cases a syringomyelia was associated with the following intramedullary tumors: one hemangioblastoma, two ependymomas, one medulloblastoma and one oligodendroglioma. In three cases gliomas were present in the subarachnoid space.

GEORGE B. HASSIN.

PRIMARY MELANOBlastOSIS OF THE LEPTOMENINGES AND BRAIN. FREDERIC J. FARNELL and JOSEPH H. GLOBUS, *Arch. Neurol. & Psychiat.* **25**:803, 1931.

A boy, aged 16, suffered for six months from repeated epileptiform attacks, headaches and fainting spells. When in the hospital he also vomited, developed drowsiness and had a temperature of 101 F. The spinal fluid was xanthochromic, contained six cells per cubic millimeter and gave a negative Wassermann reaction. Many pigmented nodes were seen on the trunk and legs. The fundus, pupils and the cranial nerves in general were normal, excepting a right supranuclear facial weakness; the tendon reflexes in the lower extremities were diminished, but a bilateral clonus was present; the Babinski sign was absent. During the following weeks the patient grew worse; the reflexes and the clonuses gradually disappeared, the cranial nerves become markedly involved (all the branches of the facial became paralyzed), the convulsions grew more frequent, and forty hours before death, a swelling of the disks began to show. Necropsy revealed grossly thickened meninges filled with black pigmented tumor cells over the ventral surface of both cerebellar lobes, pons, medulla and spinal cord, extending cephalad as far as the chiasm; in the meninges the cells somewhat resembled chromatophores. They most likely invaded the brain tissues secondarily. Other tumor cells were devoid of pigment; they were epithelioid or spindle shaped, resembling a sarcoma. Additional phenomena were cytoplasmic macroglia, and microglia, enclosing melanin, marked vacuolization of the brain and a hydrocephalus because of obliteration of the pontile and the interpeduncular cisterns. No tumor masses were present elsewhere in the body. The authors consider their case an instance of a primary melanoblastosis of the central nervous system, regardless of the presence in their patient of numerous nodes over the trunk and legs.

GEORGE B. HASSIN.

MALIGNANT LYMPHOID HYPERPLASIA IN MICE. FLORENCE MCCOY HILL, *J. Cancer Research* **14**:325, 1930.

Of 216 mice, 134 showed various forms of malignant hyperplasia regarded as leukemia, pseudoleukemia, lymphosarcoma and Hodgkin's disease. Judging from widespread invasion of organs and tissues and the destruction of normal architecture, these different forms were regarded as malignant. In all of these varieties several stages in the differentiation of the granular and nongranular cells of the blood existed side by side. It is believed that all these diseases form one single group. The growths arise from the unrestrained multiplication of a primitive cell, and the multiform appearances that are assumed later result from the secondary differentiation. The primitive cell behind all these varieties is the free reticular cell, from which all white blood cells are probably derived.

B. M. FRIED.

THE SEROLOGICAL DIAGNOSIS OF CANCER. W. M. WRIGHT and C. G. L. WOLF, *J. Cancer Research* **14**:370, 1930.

The authors were able to confirm the results of Fuchs in the diagnosis of a malignant condition on the basis of the action of serum from patients with cancer on normal fibrin. The test appears to follow Schutz's law. The reaction increases in velocity up to 45 C. and stops at 56 C. It is, within certain limits, dependent on the amount of substrates used. Other proteins of the blood cannot be utilized, but washed muscle suitably treated can be used instead of fibrin though this product loses its characteristics on standing much more easily than does fibrin itself. No cases diagnosed clinically as malignant have so far fallen into the group of cases giving a negative reaction. An alternative method of following the reaction by alcohol titration of the products of proteolysis is described.

B. M. FRIED.

EFFECT OF RADIATION ON BLOOD CHOLESTEROL IN MALIGNANT DISEASE. W. L. MATTICK and M. C. REINHARD, *J. Cancer Research* **14**:426, 1930.

Cholesterol content of the blood of twenty-five patients with cancer was determined before and after irradiation with high voltage x-rays at one half hour intervals over a period of three hours, thus supplementing a previous study on the blood of thirty-six patients with cancer. The authors could not notice any characteristic change in the cholesterol level in the whole blood of the cases studied in the three hour period. It is concluded that high voltage X or gamma radiation causes a decrease in the cholesterol content of blood from the pre-irradiation level and that this seems to constitute its most characteristic effect on this blood constituent.

B. M. FRIED.

THE DETECTION OF SMALL QUANTITIES OF LEAD IN THE TISSUES. FRANCIS CARTER WOOD, *J. Cancer Research* **14**:476, 1930.

Lead, either in the colloidal state or as an organic compound, when injected intravenously, can be demonstrated by a suitable technic in both inoculated and spontaneous tumors in animals.

B. M. FRIED.

THE INFLUENCE OF SUPRARENALIN (EPINEPHRIN) ON THE GROWTH OF CARCINOMA AND SARCOMA IN ANIMALS. K. SUGIURA and S. R. BENEDICT, *J. Cancer Research* **14**:487, 1930.

Reicher, in 1910, and some other workers produced evidences to show that the injection of epinephrine in the neighborhood of rat sarcoma and mouse carcinoma caused a central necrosis and subsequent destruction of these tumors. Sugiura and Benedict after a preliminary study of the toxicity of epinephrine on the Flexner-Jobling rat carcinoma and the Rous chicken sarcoma found that the

development of small tumors in animals is completely inhibited by repeated intratumoral injections of epinephrine, while large tumors are seldom beneficially affected by repeated injections. The repeated subcutaneous injection of this substance at a remote point does not affect the growth of rat carcinoma and chicken sarcoma. In vitro experiments have shown that epinephrine partially inhibits the proliferating ability of the Flexner-Jobling tumor whereas the Rous sarcoma remains unaffected. The authors state that if there is any possible therapeutic value of epinephrine for the treatment of cancer it is limited to local application.

B. M. FRIED.

THE EFFECT OF THE INTERNAL SECRETIONS UPON THE DIVISION ENERGY OF PARAMECIA. G. L. ROHDENBURG, J. Cancer Research **14**:509, 1930.

Spleen, liver, thymus, adrenal and testes act as a stimulant on the division energy of *Paramecia*; thyroxin, insulin, pituitary and parathyroid inhibit its division. The ovary has no influence.

B. M. FRIED.

THE EFFECT OF IRRADIATED ERGOSTEROL AND INCREASED CALCIUM ON GROWTH OF TUMOR TISSUE. ALFRED GOERNER, J. Cancer Research **14**:545, 1930.

Goerner studied the effect of irradiated ergosterol on the rate of growth of Flexner rat carcinoma and on the deposition of calcium in the tumor. The results obtained show that there is no difference in rate of tumor growth in the treated and untreated groups of animals. There likewise was no difference in the deposition of calcium in the tumors of the treated and untreated groups of animals, nor a difference in the ash content of tumors of the two series.

B. M. FRIED.

THE STATISTICS IN NEW YORK STATE FOR THE FOUR-YEAR PERIOD 1925-1928. JOHN M. SWAN, J. Cancer Research **14**:548, 1930.

Cancer is increasing in the state of New York, exclusive of New York City, but not in a constant ratio. In fact, in the last year the increase was definitely lower than that of the year before.

If there are 7,000 deaths from cancer annually in the state of New York, there must be about 21,000 cases of cancer in the state all the time, assuming that the average life of the patient with cancer is three years.

As a rule, in those communities in which cancer is looked on as a problem capable of solution, or at least of improvement, the death rate tends to be lower than in those communities in which this is not the case.

Educational activities are called for both for the medical profession and the laity throughout the state, particularly in the rural communities.

If we are able to get the physician and the layman to look on cancer as a problem to be solved, instead of as a hopeless situation, further improvement may be expected.

AUTHOR'S SUMMARY.

CARBOHYDRATE TOLERANCE AND ALKALOSIS IN MALIGNANT DISEASE. PAULINE BEREGOFF, J. Cancer Research **14**:559, 1930.

The purpose of this investigation was to observe the alterations in the blood chemistry associated with malignant disease and to determine the value of such changes in the diagnosis of human cancer.

The study was made on 281 patients with cancer, the ages of the patients ranging between 28 and 68 years. The investigation has shown that all blood specimens of patients with cancer exhibit a positive carbohydrate curve and a tendency to alkalosis. However, positive carbohydrate curves were obtained in other pathologic conditions such as hyperthyroidism, acromegalia and diabetes, but in these diseases no alkalosis was demonstrable. As an indication of the presence of malignant disease a positive carbohydrate curve is significant only in the presence of alkalosis. It would appear that the carbohydrate tolerance test and the hydrogen ion concentration are of greater value in eliminating the presence of malignant disease than of proving its existence.

B. M. FRIED.

THE LIFE HISTORY OF THE FEMALE MAMMARY GLAND IN TWO STRAINS OF ALBINO MICE. LYDIA M. GIBSON, *J. Cancer Research* **14**:570, 1930.

Since the mouse is used extensively in cancer research, and since cancer of the mammary gland is a well recognized entity in certain strains of mice, a comparative study of this organ was made. For that purpose two strains of mice were investigated; one showing a low incidence of mammary cancer in the females, and the other showing a high incidence. The study has shown that in the strain with a high incidence of mammary cancer the nipple zone in the embryo has a tendency to develop faster than that of the control strain. It is likewise inclined to show anomalies in the development of the nipple whereas the control strain has a tendency to have the mammary gland involute and fibrose in an orderly fashion as age advances. In the group with a high incidence of cancer the epithelial elements of the gland are inclined to metaplasia as age advances. The neoplasms develop in zones of chronic cystic mastitis.

AUTHOR'S SUMMARY.

THE ONCOGENIC FACTOR IN THE BLOOD AND THE ORGANS OF ANIMALS WITH INOCULATION TUMORS. T. ANARDI, *Tumori* **16**:401, 1930.

Normal rats were injected with the serum, the red corpuscles and emulsion of organs of animals that had been previously inoculated with tumor emulsion and of tumor-bearing rats whose tumor had been removed. It was shown that the transmission of tumors through the blood and the organs of animals with tumors depended on the neoplastic cells that could be found in the circulation and the parenchyma of the organs.

EMMERICH HAAM.

ACTION OF MAGNESIUM ON THE ADENOCARCINOMA OF THE RAT. A. BOLAFFI, *Tumori* **16**:420, 1930.

Neither the iodide, chloride or the phosphate of magnesium had any effect in the development of inoculation tumor in rats. The magnesium ion had no value in the treatment of cancer.

EMMERICH HAAM.

XANTHOPROTEIN, LIPOIDS AND DEXTROSE IN THE BLOOD SERUM OF PERSONS WITH CANCER. F. BENSO, *Tumori* **16**:425, 1930.

The substances giving the xanthoprotein reaction are not increased in the serum of patients with malignant tumors. The lipid substances are increased in cancer serum but become normal after the tumor is removed. The dextrose curve shows a marked difference consisting in a rapid increase and a slow decrease. Removal of the tumor turns the curve toward normal.

EMMERICH HAAM.

SOIL AND DRINKING WATER IN RELATIONSHIP TO MALIGNANT TUMORS IN ITALY. A. M. BONANNO and L. DI CORTEMIGLIA, *Tumori* **16**:433, 1930.

The varying development of sanitation in the various regions, the high child mortality and deaths from syphilis and alcoholism make the study difficult. Any influence of cosmic rays, of geologic constitution and of drinking water in the distribution of malignant tumors is denied.

EMMERICH HAAM.

PRIMARY SARCOMA OF THE PANCREAS. M. TCHIEREPNINA, *Centrabl. f. allg. Path. u. path. Anat.* **50**:50, 1930.

In addition to its rarity (Gruber has found only fifty primary sarcomas of the pancreas in the world literature), the tumor herein mentioned was of interest because of the conditions associated with it. The tumor of the head of the pancreas was the size of an orange and was surrounded by adhesions; it apparently began in a markedly cirrhotic pancreas. It was of the polymorphocellular variety, and

by extension along Wirsung's duct formed a polyp-like projection in the lumen of the duodenum. The liver was the site of an atrophic cirrhosis, and because of this, jaundice and ascites formed the background of the clinical picture. In attempting to link the various disease processes encountered, Tcherepnina considered inflammation of the pancreatic ducts primary, eventually leading to submucosal sclerosis and motor and secretory disturbances. The latter allow of toxic decomposition products in the intestinal tract, and these eventually injure the liver when brought there in the portal venous blood.

GEORGE RUKSTINAT.

MICE AS INDICATORS OF CARCINOGENIC NOXAE. O. TEUTSCHLÄNDER, *Deutsche med. Wchnschr.* **56**:2166, 1930.

A mouse, caught in a briquet factory, presented numerous squamous cell carcinomas and warts. The growths resembled the pitch warts and pitch cancers observed in some of the workers in the factory. There was pitch dust in the fur of the mouse. The spontaneous cancer in the mouse is regarded as evidence of the presence of "carcinogenic noxae" in the factory. It is suggested that mice should be kept in certain factories and other places for the purpose of acting as indicators of the presence of carcinogenic factors.

### Medicolegal Pathology

ACUTE PHOSPHORUS POISONING. ELEANOR M. HUMPHREYS and BELA HALPERT, *Am. J. Dis. Child.* **41**:354, 1931.

The risk of acute phosphorus poisoning has been decreased, but not eliminated, by the prohibition of the manufacture and sale of yellow phosphorus matches. The ingestion of fireworks containing phosphorus is a cause of acute phosphorus poisoning in children. A case of fatal phosphorus poisoning in a 4½ year old boy, traced to this source, is described. A review of the literature reveals twelve other cases. There is evidence that this figure does not give a true idea of the frequency of phosphorus poisoning caused by fireworks. The commonly implicated agent is a single type of fireworks variously known as "Son-of-a-Gun," "Devil-on-the-Walk" and "Spit-Devil."

AUTHORS' SUMMARY.

GRAVE ANEMIA IN PREGNANT WOMEN FROM BENZENE INTOXICATION. M. A. BRENDEAU, *Ann. d. méd. lég.* **11**:95, 1931.

Two women in the fifth month of pregnancy with marked anemia were employed in the same printing establishment and used xylene to clean type. One recovered after hysterectomy and transfusions of blood, and the other died following the same treatment. Chemical analysis of the xylene used revealed metaxylene and other toxic substances.

A. R. BRYANT.

LUCID INTERVAL BEFORE DEATH AFTER TWO CRANIAL INJURIES. MUTEL and DURAND, *Ann. de méd. lég.* **11**:121, 1931.

The literature is reviewed, three clinical reports are abstracted and a complete report is made on two patients who died from double cranial injuries with intervals of apparent health between the traumas.

A. R. BRYANT.

THE SIGNIFICANCE OF THE THYMIC AND THYMICOLYMPHATIC STATUS IN LEGAL MEDICINE. WIKTOR GRZYWO-DABROWSKI, *Ann. de méd. lég.* **11**:128, 1931.

Persistent thymus occurs in healthy persons as old as from 25 to 35 years of age. It is not a predisposing factor to sudden death. This statement applies

also to the thymicolymphatic state. There is no objective proof that this state, or that a persistent thymus, influences the constitution. The thymus is no longer in those committing suicide than in those who die suddenly from other causes.

FROM AUTHOR'S SUMMARY.

MEDICOLEGAL SIGNIFICANCE OF EPIDEMIC ENCEPHALITIS. GEORG STIEFLER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:227, 1931.

Criminal tendencies, particularly various sexual offenses, during the postencephalitic stages are the result of psychopathic manifestations and changes in the psychic constitution. Fourteen cases are mentioned, in which criminal actions, mostly erotic, were committed by the so-called encephalitic delinquent. Suicide and suicidal attempts and tendencies are also prevalent during the postencephalitic stages. The author discusses the practically important question of marriage of the encephalitic person, especially the validity of such a marriage if contracted during the stage of apparent latency, motivated by a definite exaggerated erotic impulse. In instances of accidental injuries involving a postencephalitic patient, one has to consider whether the disease of itself produced or directly contributed to the accident.

E. L. MILOSLAVICH.

LATE COMPLICATIONS OF POISONING BY ANILINE OR SIMILAR SUBSTANCES. ANGELO BONZANIGO, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:242, 1931.

Peculiar, transitory attacks of weakness in connection with dimming and blurring of vision, but without loss of consciousness, are a typical sequel of aniline poisoning and of diagnostic importance, but do not signify any permanent disturbance of health. Following nitrobenzol poisoning damages to the heart, various cardiac symptoms and disturbances are more prevalent and more pronounced and often of longer duration than after aniline poisoning. In poisonings with some other nitrobenzene compounds, gastro-intestinal complaints (anacidity) are marked. Poisonings with derivatives of toluene produce symptoms similar to those observed in aniline and nitrobenzene poisonings. A questionable case of retrobulbar neuritis following industrial benzene poisoning is briefly mentioned, as a report of a similar case was published in 1921 by Perlia. The industrial poisonings with aniline, nitrobenzene and similar substances may be followed by some functional disturbances that are strikingly similar to neurasthenia or neurosis.

E. L. MILOSLAVICH.

DERMOGRAPHIC EXAMINATIONS OF DEAD BODIES. G. SCHRADER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:256, 1931.

The author continued the studies of Bettmann (*Deutsche Ztschr. f. d. ges. gerichtl. Med.* **15**:1, 1930), using his original technic, on a large scale, particularly with reference to the determination of the time of death. His conclusion is that the dermatographic method does not yield any practical results in this respect.

E. L. MILOSLAVICH.

INTERPRETATION OF BLOOD STAINS. K. WALCHER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:272, 1931.

The form, size and character of blood spots and their relation to each other, may indicate the origin, the distance and the direction from which they came. In instances of penetrating injuries to the head, the blood spots may contain particles of brain, perhaps microscopic in size. Expectoration of foamy blood, as observed in cases of injuries to the lung, of aspiration of blood in basal fractures of the skull, etc., particularly if expectorated during coughing spells, leads to formation of blood bubbles on surrounding objects. When such a bubble dries, it leaves a ring of blood, while the center of the bubble, consisting of air, dries out completely.

E. L. MILOSLAVICH.

INHERITANCE OF BLOOD GROUPS. S. S. SABOLOTNY, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:277, 1931.

Investigations made on subjects native to southeast Russia prove the validity of the Bernstein theory. The practical application and medicolegal significance of blood grouping in cases of questionable paternity (mater semper certa est, pater incertus) are discussed.

E. L. MILOSLAVICH.

TWO CASES OF SUDDEN DEATH DUE TO SYPHILIS OF RESPIRATORY ORGANS. R. POHL, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:283, 1931.

The first case involved a 45 year old robust woman whose sudden death while swimming was caused by a large hemorrhage within the left thoracic cavity from a ruptured syphilitic aneurysm of the pulmonary artery, which had developed within the upper lobe of the left lung. In the second instance, a 43 year old carpenter was found dead in bed, and the autopsy revealed an acute edema of the glottis and larynx due to a diffuse gummatous infiltration of the entire larynx. The aorta and the suprarenal glands also showed syphilitic changes.

E. L. MILOSLAVICH.

EXPERIMENTAL STUDIES OF THE HIGH PLUNGE INTO WATER. FERDINAND VON NEUREITER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:305, 1931.

Theoretical studies and practical experience prove that a plunge from a great height into water will cause, owing to rebounding of the body on the surface of the water, fractures of the bones and other severe internal injuries, similarly to a high fall onto solid ground. Under certain circumstances, the surface of the water may act like an intense, blunt violence, viz., like a wide, flat and forceful impact.

E. L. MILOSLAVICH.

ACCIDENTAL INJURIES BY ELECTRICAL CURRENTS. F. PIETRUSKY, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:313, 1931.

In the last seven or eight years, 232 of 439 electrical accidents suffered by agricultural workers ended fatally. In 98 instances death was caused by a current of 220 volts and involved chiefly persons in the third decade of life. Currents of up to 500 volts killed 86 persons. In one case the electrical burn was complicated by lethal tetanus. About one year after an electrical accident involving a 24 year old man an amyotrophic lateral sclerosis developed which caused his death. Irregularity of the action of the heart, fibrillation and absence of patellar and plantar reflexes were observed as sequelae of an electrical shock in a 46 year old worker. Cardiac symptoms, such as bradycardia, tachycardia, arrhythmia, cardiac pain and myocardial weakness, are not uncommon following electrical injuries. In the majority of cases unconsciousness occurs rapidly and is followed by death shortly after. Of 204 workers who survived the accident, 134 showed more or less severe disabilities. In one case a plexus neuritis of the injured arm persisted for a year. Several times organic paralysis of the affected extremity developed. Peculiar psychic disturbances are not uncommon. In 103 cases of injury by high voltage, electrical cataract was observed seven times. In all of these cases the burns were more or less severe, and in 39 cases amputations were necessary. Interesting is the observation concerning the development of "electrical edema" in the affected extremity, a condition that was encountered in 11 instances. The cause of such an edema is not well understood. It seems that a spasm of the vessels and an abnormal permeability of their walls may be the reason. In other cases paralysis of the blood vessels, with damages to the wall of the vessel, form the main cause, but occasionally thrombosis may be the underlying factor. However, the edema, as a rule, disappears rapidly and persists rarely. Electrical

shock produces apparent death; therefore artificial resuscitation for a longer period of time is indispensable. If a sudden, powerful irritation produces an abrupt cessation of the action of the heart and of respiratory function, an artificial resuscitation at the right time will undoubtedly again revive these vital functions, since ganglion cells, the most delicate structures, regain their function up to twelve minutes after being deprived of blood supply. Since vomiting occurs during the stage of unconsciousness, one has to consider that there may be a mechanical obstruction of the upper respiratory tubes. A lumbar puncture is appropriate in instances of intracranial pressure (cerebral edema).

E. L. MILOSLAVICH.

INJURIES TO THE HEAD BY BLUNT FORCE. GEORG STRASSMANN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:327, 1931.

Traumatic hemorrhages in the depth of the white matter of the brain occur in the direction of the blow, which deforms the skull at the point of impact, leading to a sudden change in cranial volume, thereby crushing the brain substance; the wave of force ends on the opposite, contrecoup, side. Deep-seated traumatic hemorrhages of the brain are therefore always found in the direction of the action of the blunt injury and are common. Smaller hemorrhages that appear some time after the concussion of the brain occur on account of an abnormal irritability of the blood vessels due to the concussion, and disturbances of circulation, stasis and diapedesis with subsequent necrosis of the brain substance may follow. Post-traumatic apoplexy is a rare occurrence, and the great majority of the observed cases have been simple apoplexies. Pneumonic involvement of the lungs often rapidly follows injuries to the head. Purulent meningitis developed in six cases of injuries to the head by blunt force, and in each instance a fracture of the base of the skull was found. The traumatic meningitis caused death in three cases within two days after the injury, in two cases in five and eight days, respectively, and in one instance forty days after the accident. In only two of ninety-one cases of injuries to the head with concomitant hemorrhages of the brain was the skull found intact.

E. L. MILOSLAVICH.

DEATH FOLLOWING BOXING MATCH. WALDEMAR WEIMANN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:341, 1931.

In 1928, Wolff published (*Deutsche Ztschr. f. d. ges. gerichtl. Med.* **12**:392, 1928) a review of the literature on this subject. In ten of his cases death was caused by injuries to the head; in two instances a fracture of the skull was found, in seven cases there was a subdural hematoma, and in one case that ended lethally several months afterward there was hemorrhagic pachymeningitis. Weimann adds a new case, that of a 15 year old boy who died about half an hour after a blow on the chin in a boxing match. The autopsy disclosed an evenly distributed subdural hemorrhage, but no fracture of the skull or injuries to the brain. The subdural hematoma is a common cause of death in boxing.

E. L. MILOSLAVICH.

ATYPICAL GUNSHOT WOUNDS OF THE SKULL. WALDEMAR WEIMANN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:345, 1931.

In a case in which the bullet enters the cranial bone at a sharp angle, an atypical perforation of the skull may be observed. In such an instance, the external table may be raised in splinters by the penetrating bullet, producing a crater-like, funnel-shaped hole, its wall slanting outward, thus closely resembling a bullet outlet. Several pictures illustrate this mechanism clearly.

E. L. MILOSLAVICH.



HISTOPATHOLOGY OF SODIUM HYDROXIDE POISONING. L. JANKOVICH, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:352, 1931.

The course of sodium hydroxide poisoning cannot be divided into certain stages that follow one another as in most other pathologic processes; various stages of the changes in the tissues are found concomitantly; for example, necrotic areas are often seen surrounded by granulation tissue. In instances of severe sodium hydroxide poisoning, the following picture is commonly observed: During the first few days after the poisoning, the eschars detach, but rarely mechanically. The inflammatory demarcation starts after a few hours and continues until it reaches its full development at the end of the first week. Then follows, between the seventh and the ninth days, an almost complete separation of the necrotic tissue; at this time large ulcerations appear. But small necrotic areas may be seen sloughing even to the end of the fourth or the fifth week. During the third or fourth week, the ulcers may become inflamed, delaying their healing for two months or more. In the second week, granulation tissue surrounds the ulcers and the larger defects, and a few days later fibroblasts and even fibrils of connective tissue develop. Fibrillar connective tissue is found in the third week, and fibrous scar tissue at the end of the fourth week. The strictures of the esophagus during the first two weeks are only spastic or inflammatory; real strictures occur after this period of time. A final healing of the ulcerous process may be prolonged for months, and a complete epithelization may fail to develop until years afterward. The delay in the healing process depends not only on the depth of the corrosive penetration, the extent of the formation of the ulcer and the emaciation of the patient, but also on the increasing obliteration of the small blood vessels near the ulcers.

E. L. MILOSLAVICH.

DETECTION OF ALCOHOL AT AUTOPSY. OTTO SCHMIDT, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:373, 1931.

A new simple qualitative test for the purpose of ascertaining rapidly during postmortem examination whether or not alcohol is present in the body is described. The test is based on reduction of an alkaline potassium permanganate solution by alcoholic vapors. The red color of permanganate turns green, and then a brown sediment of manganese dioxide appears. The test is sensitive and will detect alcohol in solutions of 1:20,000.

E. L. MILOSLAVICH.

INSULIN POISONING AS A CAUSE OF AN AUTOMOBILE ACCIDENT. J. FOG and MAX SCHMIDT, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:376, 1931.

A 25 year old chauffeur who had been treated for a long time with insulin because of diabetes mellitus collided, for no explainable reason, with a truck. He was completely confused mentally and showed intermittent aphasia and agraphia and alexia. The blood sugar was 0.075 per cent. In other instances of insulin poisoning, cerebral symptoms, such as epileptiform convulsions, diplopia and hemiplegia, are not uncommon. The question is raised as to whether a diabetic patient under treatment with insulin should be allowed to drive an automobile, since Sonne proved that about 96 per cent of patients using insulin are subject to attacks of insulin poisoning sooner or later.

E. L. MILOSLAVICH.

RAPE AND DEFORMITY OF MALE SEXUAL ORGANS. A. SCHACKWITZ, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:384, 1931.

A charge of rape on a 14 year old girl was brought against a man who was suffering with an anatomically peculiar deformity of the penis due to a bilateral inguinal hernia, with secondary inflammatory changes and marked edema. The physical examination, which is described in every detail, proved that sexual intercourse could not and did not occur.

E. L. MILOSLAVICH.

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, March 26, 1931*

LEILA CHARLTON KNOX, *President, in the Chair*

### A CASE OF CONGENITAL ABSENCE OF ONE KIDNEY, WITH URETHRORECTAL FISTULA, URETHRAL STONE AND CONCOMITANT MEASLES. LAWRENCE W. SMITH.

A case presenting relatively unusual urogenital anomalies and concomitant measles is reported through the kindness of Dr. Meredith F. Campbell, Dr. John D. Lyttle and Dr. Jerome M. Kohn. No strictly comparable case has been found in the literature. A boy of 5 years had passed fecal material in the urine from birth. At 10 months of age he had been operated on for stone in the bladder, and a persistent pyuria had developed, with digestive disturbance and malnutrition. He was again operated on for vesical stone six months before the present admission. There were found a urethrorectal fistula with fecal mucopus and two stones in the bladder, a normal right renal pelvis and a left ureter that became lost in the large globular shadow of the colon. There was likewise a partial stricture of the rectum. Treatment was directed at clearing up the urinary infection so that operative measures could be attempted, when the boy developed typical measles. This passed uneventfully, and then suddenly signs of meningeal irritation and uremia appeared, with nonprotein nitrogen of 85 mg., and leukocytosis of 38,000, but a normal spinal fluid, except for slightly increased pressure. The patient died within twelve hours.

Post mortem were found urethrorectal fistula, urethral stone, vesical stones, chronic cystitis, chronic pyelitis (right), left renal aplasia, anomalous left ureter, megarectum, chronic proctitis, rectal stricture, terminal bronchopneumonia and edema of the brain. A review of the embryology explains the probable development of the anomalies of the urinary tract. The literature contains about 400 cases of renal aplasia, and no cases were noted with the combination of the lesions found.

#### DISCUSSION

H. S. JECK (by invitation): This is a unique case. I have never observed a recto-urethral fistula of congenital origin, to say nothing of the missing kidney. I have seen only two cases that I believe were cases of congenital absence of one kidney. Some ten years ago I operated on a man about 45 years of age, with a diagnosis of tuberculosis of the left kidney. When the left renal region was exposed, a careful search failed to reveal the kidney, ureter or renal vessels. At the present time there is a patient in the Cornell Clinic who evidently has no left kidney. Repeated cystoscopic examinations, three attempted intravenous pyelograms and roentgen examination have failed to reveal a kidney or a ureter on the left side. I think the case presented is another reminder to look for a kidney that is capable of sustaining life, before removing a diseased kidney.

MEREDITH F. CAMPBELL (by invitation): I thought urograms of the case would be of interest. The first shows the shadow of the two stones, estimated to be about 1 cm. in diameter, lodged in the posterior urethra. The second, a cystogram, shows a small, contracted bladder, pushed to the right by an enormously dilated rectum. There is also an enormous dilatation of the posterior urethra, where the stones are lodged. The third picture is of the gastro-intestinal roentgen series, showing the enormous rectum and the infundibular contraction of the rectum

at the site of the stricture. From the fourth picture the urographic diagnosis of solitary kidney was made. Here a catheter had been inserted to the right kidney and a second catheter passed through the fistula in the urethra into the rectum. Twelve per cent sodium iodide was injected through both catheters, and the outline of the right kidney and the enormously enlarged rectum were demonstrated. At the time of cystoscopy there was an intense cystitis, and when the orifice on the left side was found impassable, a large dose of indigocarmine was given, which appeared in normal concentration and in normal time from the right kidney, but which did not appear from the left side, nor in the rectum.

An operation to attempt the closure of the fistula was anticipated, but measles developed.

A couple of years ago I reported a series of nine cases of solitary kidney from the Urological Service of Bellevue Hospital, one of these being a clinical case, and eight, cases that came to autopsy. These eight cases occurred in 13,000 autopsies. Why the left kidney was so frequently absent I do not know, but the ratio of 3:1 was borne out in these studies.

JEROME L. KOHN: While this case may be of special interest because of the urologic findings, it is of equal interest on account of the measles. This was a case of mild measles with sudden death without pulmonary complication. Patients dying of measles can be divided into two groups: First: those who are very ill from the onset and who die within a few days. This type of measles, of course, occurs in regions where there never has been any measles or where there has been none for a long time. Dr. Denton described cases of this type in the Canal Zone. Second: patients who have extensive pulmonary infiltrations and who rarely die before the tenth day after the onset of symptoms. I cannot entirely agree that the pulmonary changes were terminal. The lungs showed the usual peribronchial infiltration found in necropsies in cases of measles. A careful study of the lung in this case might give a clue to whether the pulmonary changes in measles are caused by the measles virus or are due to secondary infection. Clinically the child had no pneumonia.

#### CANCER OF THE THYROID GLAND. HOWARD M. CLUTE and SHIELDS WARREN (by invitation).

The material for this study consists of all cases of malignant tumors of the thyroid gland in the Lahey Clinic, Boston, from 1916 to Jan. 1, 1930, inclusive. This includes 34 cases of adenoma with invasion of the blood vessels, which were found by going through 1,114 old slides not previously examined for the presence of such invasion. The total number of cases is 187. These occurred among 6,535 cases in which operation was performed for disease of the thyroid gland, an incidence of malignancy in this material of 2.86 per cent.

*Grouping of Cases.*—Clinical grouping is considered as follows: group I—low or potential malignancy; group II—moderate malignancy, with a hope of cure; group III—marked malignancy, in which there is practically no hope of cure. A histologic classification is made, which is found to follow very closely this clinical grouping, as follows: group I—adenoma with invasion of blood vessels; group II, papillary cystadenoma. In group II is adenocarcinoma (1) of the papillary type and (2) of the alveolar type. In group III there are (1) squamous cell carcinoma; (2) small cell carcinoma (*a*) of the compact type and (*b*) of the diffuse type; (3) giant cell sarcoma, and (4) a group in which is placed 1 probable case of fibrosarcoma. A clinical discussion of the members of each group is given, and the histologic observations of each type detailed and pictured.

In group I—low or potential malignancy—there were 133 cases, with a mortality of 4.5 per cent. In group II there were 21 cases, with a mortality of 23.9 per cent. In group III there were 33 cases, with a mortality of 82 per cent, making a total of 187 cases, with a mortality of 20.3 per cent. Of the 187 cases, only 7 were lost in the follow-up, 180 being followed for one year or more since operation.

This classification of tumors of the thyroid gland is a simple one to follow histologically, and from the clinical point of view it offers a distinct advantage in the grouping of cases and the methods of treatment for cases and the prognosis in such cases.

## DISCUSSION

LAWRENCE W. SMITH: It is with unusual interest that I have listened to this paper, as it happens that it records, among other things, the end-results in a series of cases in which I made a good many of the original diagnoses. It is also stimulating to have such a check-up on one's work, and in no organization with which I am familiar has the follow-up work been so effectively managed as it has in the Lahey Clinic. In our original observations we followed an arbitrary and rather unorthodox classification, which has been presented this evening in somewhat modified form, as we anticipated at that time would be necessary. But it is interesting to note how much more satisfactory a classification it has turned out to be than the more usual terminology.

Since that time I have had the opportunity of examining all the thyroid glands from the New York Hospital, and during this interval have studied some 46 additional cases of malignant tumors. Our data are not complete enough to include any clinical reports at this time. In general, however, the cases have fallen into these same major groups, and their clinical course in most instances has corresponded to the expectancy in each group, as gleaned from Dr. Clute's and my original studies, and as confirmed by this additional report. Of these cases, as in the Lahey series, the majority, or 33 cases, fall into their first two groups, those of low grade or moderate malignancy. They are about equally divided into two types: 18 were of the malignant adenoma or alveolar type with invasion of blood vessels, and 15 were of the papillary type with capsular invasion, including 2 cases of definitely aberrant thyroid gland. One of the others falls into the thyroglossal duct type, and the remaining 11 fall into the extremely malignant group, 8 being of the small cell type and 3 of the giant cell pseudosarcoma type.

The malignant adenoma group requires little or no discussion other than to concur in the opinion regarding the value of vascular invasion as a criterion for malignancy, and to hazard again the theory that the papillary type is specifically derived from the lateral anlage of the gland embryologically.

The small cell group is to my mind the one most worthy of comment. The paper just presented includes 17 of the compact type and 8 of the diffuse type. Of the patients in these cases, 21 are dead, 1 has a recurrence, and 3 are alive after one, one and three-quarters, and two and two-thirds years. In one case x-rays were of value in "curing" the tumor locally, and the patient lived for three and a half years with local recurrence, dying, however, presumably of retroperitoneal metastases. Our 8 patients with comparable cases I believe are all dead, although there is some uncertainty regarding one who is "lost."

I am much interested in Dr. Warren's separation of these tumors into two groups, the "compact" one, which he is inclined to call carcinoma, and the "diffuse," which I suspect he feels may well be lymphoid. We have encountered the same difficulties in our cases, and are somewhat divided in our opinions regarding their histogenesis.

Certain facts stand out sharply; these cases occur almost entirely in the sixth decade; they usually develop in a preexisting thyroid adenoma; the tumors grow rapidly, and the patient is usually dead within from six to eight months after he is first seen. The tumors are, as far as we have been able to find out, absolutely radio-resistant. For these reasons, especially their resistance to radiation, I am inclined to feel that we are dealing with carcinoma rather than lymphosarcoma, although I am free to confess that certain of them are impossible to prove histologically. Dr. Clute's own case, which responded temporarily to radiation, might well establish the fact that true lymphosarcoma may occur. One other case I have seen which microscopically presented the typical picture of a reticular cell type of lymphosarcoma, but most of them lack the histologic criteria for determining their histogenesis.

The chief point of difficulty is their differentiation from cases of Riedel's struma, a distinction of some practical importance to the patient. And it is perfectly possible that the apparent chronic inflammatory process of ligneous thyroiditis may become neoplastic, sometimes developing into a lymphosarcoma. It is difficult to accept this idea with any enthusiasm, as I can think of no strictly comparable condition to support the contention.

We, too, have found cases of "giant cell" tumors in which it is extremely difficult to eliminate "fibrosarcoma" as a diagnosis. It is quite possible that such tumors may occur, but it is my firm belief that an epithelial origin can be traced if one searches long and hard enough. It is a good deal like a garden overrun with weeds, in which the flowers are gradually choked out. So, in these tumors, it may be that the stroma outgrows the original epithelium, as in the so-called carcinosarcoma of the uterus, breast, etc. It is interesting to note in this group of seven cases, a case in which the patient lived two years, and another in which the patient is still alive two and a half years postoperatively. In the New York Hospital series, all 3 patients died within a few months of the recognition of the condition.

May I say again how indebted I am to Dr. Clute and Dr. Warren for bringing my knowledge of these cases of thyroid tumors up to date? I am, of course, pleased that this general method of classification has worked out satisfactorily. I feel that we are gaining tremendously in our knowledge of the natural history of malignant disease of the thyroid by the presentation of such complete data as have been given tonight, and particularly of such a large number of cases, and I look forward in the near future to the time when the mystery of the histogenesis of these extremely malignant small round cell tumors is solved.

LLOYD F. CRAVER (by invitation): I have been interested and instructed to hear the paper of Dr. Clute and Dr. Warren, and particularly interested to know that they have had decidedly good results from x-ray treatment in certain of their groups of cases. It seems to me that in thyroid carcinoma one meets with certain surprises in radiation, inasmuch as some of the tumors which are so highly cellular, which one would expect from the general laws of radiation would be radiosensitive, apparently do not prove to be so, and others, such as the papillary adenomas and the papillary adenocarcinomas, which have a more adult structure, and which one would not perhaps *a priori* expect to be radiosensitive, do appear to be radiosensitive; in fact, some of them are quite so. I have in mind two illustrative cases. One of these occurred in a woman about 30 years old, who had a papillary adenocarcinoma, which was incompletely removed. She came to us five or six years ago and was treated with high voltage x-rays. Numerous cysts formed and were aspirated from time to time. Finally, after four or five aspirations, the cysts stopped filling up with fluid, and there began to be a definite regression of the papillary adenocarcinoma. She has failed to return now for about three years, except for one time about a year ago when I saw her. At that time she still had a small nodular mass in the neck, but she had gained about 25 pounds (11 Kg.) and had no evidence of any activity of the thyroid carcinoma, so that her tumor was a type which *a priori* we would expect not to be radiosensitive, but which proved to be so, showing a definite regression following radiation and a growth restraint that has lasted.

Another patient, a man about 50, had a mass the size of a fist arising from the right lobe of the thyroid—hard and solid. This was treated extremely heavily with high voltage x-rays, but it did not regress. We sent him to a surgeon in New York who specializes in thyroid surgery. He removed the mass, but missed some nodes which were above the tumor in the side of the neck, and which we knew were there. We sent the patient back for a subsequent operation for the removal of the nodes. Following that operation the examination of the tissue showed that it was a highly cellular carcinoma made up of rather solid sheets of cells which we would expect from the microscopic structure might be radiosensitive, and yet it had proved not to be so. However, a few months after

the second operation a number of small, shotty metastases appeared in the same side of the neck, and following radiation, they disappeared almost as rapidly as a lymphosarcoma, so that in that one patient we have an illustration of the surprises that one meets when radiating thyroid carcinoma.

On the whole, I have been rather encouraged by the radiation treatment of thyroids. We get at the Memorial Hospital a great number of patients with nodular goiters who have refused operation, or who have mild toxic symptoms. The standard surgical teaching on this subject is that the treatment of choice is the surgical removal of an adenoma, and I think that means to the average man that any nodule in the thyroid should be removed. There is a widespread confusion in terms between adenoma and the mere nodular goiter, which represents probably the changes arising from various functional cycles of hyperplasia and involution. I do not know how anyone is going to distinguish clinically in a great number of these cases between a nodular thyroid and the true adenoma that may be a precursor of cancer. I think the routine radiation of many of these nodular thyroids, as we have been carrying it out, may be of value not only in relieving toxic symptoms—and occasionally we get surprises in the disappearance of the nodules—but in exercising restraint on the growth of a possible incipient thyroid carcinoma in patients who would not consent to be operated on, and in whom otherwise carcinoma might develop.

CARCINOMA OF SUPRARENAL CORTEX WITH UNUSUAL METASTASES. CHARLES T. OLCOTT.

A case was presented of a man of 38 who had epigastric pain and general weakness without definite localizing symptoms, also diabetes mellitus. Biopsy of tissue from a left supraclavicular node showed adenocarcinoma, but the primary focus was not recognized, although it was considered gastro-intestinal, or possibly suprarenal.

At autopsy, a large mass of whitish tissue was found to surround the left suprarenal and to extend to the stomach, pancreas and spleen. There was a fungating mass projecting into the gastric lumen just below the cardia, and here the mucosa was slightly eroded. The gastric wall in the same region was greatly thickened. Other masses of tumor extended to the mesenteric nodes as far as the cecum. The mediastinal nodes were also involved, as were those in the left supraclavicular region. The liver and the lungs contained small metastases. The tumor was found to invade the wall and lumen of the right iliac vein and vena cava. Numerous shiny nodules were seen in the peritoneum and pleura. Microscopic slides were shown, including one in which there was a transition from normal to cancerous cells in the same cell column of the zona fasciculata of the left suprarenal. The tumor cells were large and vacuolated. On frozen section these contained lipid material with the staining reaction of that found in the suprarenal cortex. Similar cells were found in all layers of the gastric wall, except the superficial portion of the mucosa. In other respects, the gastric glands were normal and showed no suggestion of primary tumor formation. Although the large size of the tumor in the stomach militated against its being a secondary growth there, the tumor alveoli even in the stomach resembled those of the suprarenal cortex, and the large cells and multiple metastases confirmed the primary suprarenal origin of this carcinoma.

Nineteen cases of suprarenal cortical tumor were collected from the literature. Vascular and visceral metastases were noted in fourteen. The fascicular arrangement and the presence of lipid droplets were characteristic.

PSITTACOSIS: POSTMORTEM EXAMINATION OF A CASE, INCLUDING STUDIES OF THE SPINAL CORD. S. H. POLAYES and M. LEDERER.

The case is reported because as far as could be ascertained, it is the first case of psittacosis in New York City in which the diagnosis was confirmed both by bacteriologic and postmortem examination, and because the case permitted studies

of the changes in the spinal cord. A Jewish housewife, 51 years of age, was admitted to the Jewish Hospital of Brooklyn on the private service of Dr. M. Rabinowitz on Feb. 5, 1931, with physical signs and symptoms of pneumonia. Vague prodromal symptoms had begun a week before admission. In spite of the evident bilateral pulmonary consolidation on admission, the patient did not complain of any pains in the chest, and the expectoration was scant. A pleural rub was not heard until two days after admission. At this time, also, the creeping nature of the consolidation was noted in the left lung when the base, which was originally involved, became more resonant, while the process ascended to the apex. The other features of this case were the typhoid state of the patient, the abdominal distention and diarrhea of foul, watery stools, and the delirium, which persisted until her death on the eleventh day after admission. During the course of the illness it was learned that the patient, as well as several others of her family, had contact with sick parrots, which one of her brothers had recently brought from Havana. Some of these parrots died soon after importation. One of her brothers, who received a pair of the diseased parrots, died of what was at first considered pneumonia, but later believed to be psittacosis. The entire family, including the patient whose case is described now, had attended the funeral of the first victim. Shortly after that the patient and three others of the family contracted the disease. Of the latter three, two made an uneventful recovery, while the convalescence of the third was prolonged by a complicating pulmonary embolus.

*Laboratory Data.*—The urine showed traces of albumin, and occasional red and white blood cells. Examination of the blood showed: 4,490,000 red blood cells per cubic millimeter, with 0.5 per cent reticulated cells; 10,800 white blood cells per cubic millimeter, with 86.5 per cent polymorphonuclear neutrophils of which 45 per cent were nonsegmented forms, 11 per cent lymphocytes, 1.5 per cent monocytes, 0.5 per cent neutrophilic myelocytes and 0.5 per cent metamyelocytes; 150,000 platelets per cubic millimeter; hemoglobin (Dare) 64 per cent; color index, 0.7 per cent; bleeding time, 10 seconds; coagulation time, 10 minutes. Repeated examination gave similar results. A blood culture on February 6 was reported sterile after three days' incubation. The spinal fluid, released under increased pressure, was opalescent owing to the presence of numerous red blood cells; albumin and globulin were present; Fehling's solution was reduced; the Wassermann reaction was negative; colloid gold was not reduced.

*Bacteriologic Examination.*—Tissues from the lung, liver and spleen, secured under aseptic precautions, were submitted to Dr. G. P. Berry of the Rockefeller Institute for bacteriologic study. Dr. Berry reported that he identified and isolated the psittacosis virus from each of these tissues. He also isolated the virus from the sputum of one of the survivors and from the liver and spleen of the last remaining parrot received from the home of one of the other survivors.

In the postmortem examination the changes in the lung and spinal cord were emphasized. (Examination of the brain was not permitted.)

*Pulmonary Changes.*—So much controversy is centered about the resemblance between psittacosis and influenzal pneumonitis that the authors considered it important to tabulate the points of difference between these two conditions (see table).

Other less constant changes which may be of aid in recognizing the pneumonitis of psittacosis are the dark purple-blue color of the lung, the relative absence of pleural exudate and the occurrence of small subpleural hemorrhages.

*Changes in the Spinal Cord.*—It is pointed out that the clinical signs that are so common in this disease (photophobia, lethargy, stupor, sluggish speech, slow response, muttering delirium, backache, ataxia, incontinence and even localizing signs such as facial immobility, muscle jerks, etc.) indicate anatomic changes in the central nervous system. The literature contains little information regarding these changes. The outstanding findings in some of the cases studied are: congestion, edema, foci of softening, hemorrhagic pachymeningitis and circumscribed areas of myelin degeneration. The findings in the spinal cord of the case reported

here are: an increase in the glial cells of the anterior horn, chromatolysis, distortion of shape and eccentric displacement of nuclei of the neurons of the anterior horn, perivascular round cell infiltration, marked congestion of blood vessels, numerous punctate hemorrhagic areas and marked fat replacement in the anterior horn cells. Although these changes are definite, they cannot be considered characteristic of this disease, since similar changes in the spinal cord may occur in toxic states other than those associated with psittacosis. A plea is made for further studies of the changes in the central nervous system in psittacosis.

The history, epidemiology and bacteriology of psittacosis are also reviewed and the pertinent facts emphasized.

#### DISCUSSION

G. P. BERRY (by invitation): The pathologic lesion of the lungs that Dr. Polayes has described is really quite unique, and as he pointed out, differs distinctly from influenza, as it does also from other pneumonic processes. The lesion can be reproduced in monkeys of the species *Macacus rhesus*, and in these animals there is a chance to follow the evolution of the pulmonary changes.

#### *Points of Difference Between Psittacosis and Influenza*

Points of Difference	Psittacosis	Influenza
Type of consolidation.....	Lobar .....	Nodular, even when areas of consolidation become confluent
Cells lining the alveoli.....	Show marked hyperplasia and hypertrophy	Show no marked change
Type of cellular exudate....	Polymorphonuclear cells are absent or scarce—exudate consists of mononuclear cells, plasma cells and desquamated epithelial cells.	Numerous polymorphonuclear cells—occasionally to extent of suppuration
Relation of bronchi to areas of consolidation	None; bronchioles involved by extension of process from surrounding lung tissue	Definite; capillary bronchitis with bronchopneumonia
Deposit of fibrin.....	Very abundant throughout...	Scarce, except near involved bronchioles
Character of hemorrhagic lesion	Associated with capillary thrombosis and severe necrosis of lung tissue	Not associated with either
Bacteria in the lung tissue..	Scarce or absent, except in areas of secondary infection	In large numbers throughout the lung

Monkeys can be inoculated in the trachea or in the nose. After forty-eight hours the experimental pneumonia begins to develop. It runs a course for a period of about ten days, and then undergoes rapid resolution. Most of the animals recover, but a few die from a spreading pneumonia. The first abnormality that one notices in sections from monkeys killed after forty-eight hours is a slight edema of the alveolar walls, beginning at the hilus and spreading toward the periphery. As the edema becomes more pronounced, small amounts of fibrin are laid down, and one finds the alveolar walls becoming infiltrated with cells. At first, in some places, there are some polymorphonuclear cells, but as the lesion grows older, only various types of mononuclear cells are to be seen. The infiltration continues, and at the same time the alveoli become more distended with albuminous material, fibrin and large cells with single nuclei. The exact nature of these cells is obscure; undoubtedly many of them are desquamated epithelial cells from the alveolar wall, and probably others belong to the "mononuclear series." Presently the entire lung becomes very cellular; the alveolar walls are tremendously distended by these cells and the alveoli filled with them. Often it is almost impossible to distinguish the alveolar walls. Thrombosis of small vessels occurs, and small hemorrhages are scattered through the congested tissue. Necrosis of the alveolar walls follows in the thrombosed areas. On gross section, at this



stage of the process, the lung shows a smooth, rather dry, homogeneous structure, such as was present in Dr. Polayes' case. Although the distribution is often lobar, the appearance is unlike that of lobar pneumonia. In a way, the psittacotic lung resembles a glandular structure, meaty, smooth, a little translucent, but not granular. It is surprising that such an extensive process can clear up in two or three days. The roentgen shadow may completely disappear in this time. The process heals without fibrosis; the cells disappear; a moderate degree of emphysema may develop, but in a short time the lung tissue becomes quite normal again.

The literature contains few studies of the central nervous system in patients with psittacosis. Only a few reports of autopsies include neuropathologic observations. In view of the many symptoms and signs of the disease referable to the central nervous system, and of the neurotropic properties of the virus under experimental conditions, further observations are indicated. In animals inoculated intracerebrally a meningo-encephalitis develops. The meningeal exudate consists of polymorphonuclear and mononuclear cells. Some perivascular infiltration extends into the brain and cord from the meninges, and there are a few scattered hemorrhages. Nerve cell changes, similar to those described by Dr. Polayes, have also been encountered. It seems hardly necessary to point out the fact that the lungs and brains of such experimentally infected animals are sterile for all the ordinary types of bacteria.

## PATHOLOGICAL SOCIETY OF PHILADELPHIA

*Regular Meeting, May 14, 1931*

BALDUIN LUCKÉ, *President, in the Chair*

### HODGKIN'S DISEASE OF BONE MARROW AND SPLEEN WITHOUT APPARENT INVOLVEMENT OF LYMPH NODES. E. B. KRUMBHAAR.

A white painter, aged 55, was admitted to the Philadelphia General Hospital (service of Dr. Boston) complaining of weakness of the legs. He had had dyspnea, palpitation, nocturia, anorexia and constipation for several months. He had had scarlet fever and gonorrhea and was a moderate user of alcohol. He gave no history of having had syphilis. Physical examination showed nothing abnormal, except for pallor, ascites and a greatly enlarged spleen. No lymph nodes were palpable either before or after death. The Wassermann reaction was negative. There were much albumin and many casts in the urine. Anemia was marked. The leukocyte count was 8,200; later, 3,700 (polymorphonuclears, 54 per cent; lymphocytes, 43 per cent).

At autopsy performed by Dr. Palomeque, the spleen weighed 1,090 Gm., the surface cut with increased resistance and was pinkish red, firm and not congested. The malpighian follicles were not visible.

The liver weighed 2,190 Gm. It was congested and diffusely fibrosed, with many small whitish areas that were not explained by the section taken.

The bone marrow, both femoral and tibial, was greatly hyperplastic. No enlarged lymph nodes of any kind were found on careful examination.

Histologically, the spleen and both bone marrows showed extensive involvement by the characteristic lesions of Hodgkin's disease. The lymphoid follicles of the spleen and of the gastro-intestinal mucosa were small and showed no noteworthy changes. A few Hodgkin's cells were found in the sinuses of the liver. As no grossly abnormal lymph nodes were found, no lymph node tissue was taken for sectioning.

Without proof that is unequivocal, this is considered a case of Hodgkin's disease of the spleen and bone marrow without demonstrable involvement of lymph nodes, a condition that has not apparently been previously reported.

This article will be published in full in the *American Journal of Medical Sciences*.

A CASE OF HODGKIN'S DISEASE OF UNUSUAL DURATION AND EXTENSION.  
MAX M. STRUMIA.

This case is interesting because of the long duration, the large amount of roentgen treatment applied, and especially for the unusual extension and quality of the lesions found at autopsy, which was performed at the Misericordia Hospital. I am indebted to Dr. Pfahler and to Dr. Bertin for the clinical notes of the case.

The patient, a woman, first noticed swelling of the neck and axilla in December, 1920, when she was 15 years of age. There are reasons to believe, however, that the disease probably started about one year before. From that time until death, which occurred in the month of February, 1928, there were an uninterrupted series of glandular swellings of the cervical, axillary, inguinal, prevertebral and mediastinal regions, enlargement of the spleen and liver and tumor formations over the left sacro-iliac joints and other portions of the skeleton. In addition, roentgenograms revealed severe absorption of the head of the left femur, of the right portion of the sacrum and of the pelvic bones. In September, 1924, the patient had three hemiplegic attacks with loss of power and sensation in the right arm and leg and loss of speech. These lesions rapidly disappeared under treatment with the x-rays. A severe herpes zoster of the left side of the face and forehead appeared in October, 1926, which healed under treatment with the x-rays, leaving a deep-seated, atrophic scar.

The blood picture was carefully studied, and never offered anything worthy of notice, save for an occasional shower of Rieder cells. There was also a mild, progressive secondary anemia.

At autopsy severe emaciation was found (weight 80 pounds [36.3 Kg.]). The superficial lymph nodes were all small, hard and fibrous. There was pericardial, pleural and peritoneal effusion. The pericardium and the myocardium showed several tumorous infiltrations, about from 4 to 6 mm. in diameter, hard and pale gray. The right lung was extensively infiltrated with large bands of hard, fibrous tissue. In addition the pleura was peppered with small, pale, yellowish nodes, from 3 to 5 mm. in diameter. In the left lung the lesions were even more advanced, with the formation of deep-seated, retracted scars, with adhesions. The spleen weighed 350 Gm. Both the surface and cut sections showed numerous irregular, white, hard nodes, with complete loss of the splenic structure. The liver weighed 1,500 Gm. The consistency was greatly increased. The surface was peppered with nodes, which had partly fused together, and at times seemed to form a diffuse infiltration around the bile ducts. The right lobe was almost entirely substituted by large, white, lardlike masses. Similar, but less extensive, tumorous infiltration was found in both kidneys and in both suprarenal glands.

The mediastinal lymph nodes formed a hard, gray, fibrosed mass, 10 by 4 by 0.08 cm. Other internal lymph nodes were small, hard and fibrosed—some of them with soft, necrotic centers.

In addition, the head of the left femur, the bones of the pelvis and practically all of the vertebral bodies appeared invaded in part by a hard, white, fibrous mass, with considerable destruction of bone.

The bone marrow of the ribs was very active; that of the femur was moderately hyperplastic. The bone marrow of the tibia was fatty.

Microscopic examination revealed that the tumorous masses and infiltrations of the various organs were formed mostly of hard, fibrous connective tissue, with extensive areas of hyalinization and degeneration, and occasional groups of cells, usually at the periphery of the lesions. These infiltrations were composed mostly of lymphocytes, monocytes, a few eosinophils and occasional typical Dorothy Reed giant cells.

CAROTINOID PIGMENTS IN BIRDS. E. P. CORSON-WHITE.

Protocols on forty-four birds with deeply stained fat were found scattered through the postmortem records of the Philadelphia Zoological Garden from 1904 to 1931. The color ranged from orange-yellow to orange-red. In thirty-

nine instances no mention was made of pigmentation in any location other than the fat; in four it was said to be present in the skin and fat, and in one in the skin, claws, beak and fat. The greatest intensity of color appeared in those regions without feathers or where the plumage was thin.

The birds were, as a rule, markedly fatter than the average of the species: three were described as markedly obese, one as extremely obese, thirty-two as fat and the remainder as well nourished. In only nine was there sufficient gross pathologic change found to account adequately for death. Of these, four had hemorrhagic enteritis, one arteriosclerosis and interstitial nephritis, one acute necrotizing enteritis, two hyperplasia of the thyroid gland with colloid and one tuberculosis of the liver and the spleen. In eleven the cause of death was not determined; in sixteen it was due to injury, i. e., to attacks by cage mates; the remainder showed mild lesions insufficient to cause death.

Analysis of the fat and tissues showed the pigments to be a xanthophyll, similar to that found in the hen's egg. The twenty-four hour ration of these birds yielded carotin and xanthophyll (methods: Tsweet's chromatograph method,<sup>1</sup> the methods of Willstätter and Mieg<sup>2</sup> and Willstätter and Stoll<sup>3</sup> and the spectroscopic method<sup>4</sup>). An effort to study the distribution of the pigment in the various tissues was attempted chemically, but because of the small amount of material this attempt was abandoned and an effort made to stain blocks of tissue, with use of the Nile blue sulphate staining methods developed by Dolley and Guthrie<sup>5</sup> in their studies of the carotinoids in animal tissues and especially in their study of the yellow pigments of the central nervous system. They identified these pigments as carotinoids, and found that they could be increased or decreased at will by varying the amount of carotinoids in the diet, and definitely showed that they were not, as previously thought, a product of wear and tear. They also observed that any drug or other factor that depressed the metabolism exaggerated the deposition of the pigment. By their method neutral fat was stained a salmon red and the carotinoid granules a deep blue. It is known that the pigments appear as intercellular and intracellular substances, possibly as an amorphous substance, but usually coloring the true fat or bound to the protein. It is questionable if they ever occur in pure condition in animal tissues. They never appear as crystals. The studies of Smith<sup>6</sup> later showed that the Nile blue sulphate, instead of particularizing the carotinoid pigments, really differentiated the neutral fats and the fatty acids. Connor,<sup>7</sup> in an investigation of all the so-called differentiating stains for lipochromes, concluded that if a yellow pigment crystallized in a tissue treated with a weak alcoholic solution of potassium hydrate and formaldehyde or lost its color in a strong solution of ferric chloride or was dissolved in a fat solvent, it was probably a lipochrome. The results with sections were therefore discouraging. The pigments seldom appear as granules, except in the skin. Elsewhere they are usually in solution in a lipid and are not visible as a particulate substance.

The toxicity of the pigments has been investigated by two observers, Wells and Hedenberg,<sup>8</sup> who injected pure carotinoids intraperitoneally into guinea-pigs, and by Hess and Myers,<sup>9</sup> who injected them subcutaneously into infants, with

1. Tsweet, M.: Ber. botan. Gesellsch. **24**:284, 1906.

2. Willstätter, R., and Mieg, W.: Ann. d. Chem. **1**:355, 1907.

3. Willstätter, R., and Stoll, A.: Untersuchungen über Chlorophyll Methoden und Ergebnisse, Berlin, Julius Springer, 1913.

4. Kohl, F. G.: Untersuchungen über das Carotin und seine physiologische Bedeutung in den Pflanzen, Leipzig, 1902, p. 165.

5. Dolley, D. H., and Guthrie, F. V.: J. M. Research **40**:295, 1919; **42**:289, 1921.

6. Smith, J. L.: J. Path. & Bact. **12**:1, 1908.

7. Connor, C. L.: Studies on Lipochromes, Am. J. Path. **21**:235, 1928.

8. Wells, H. G., and Hedenburg, O. F.: J. Biol. Chem. **27**:213, 1916.

9. Hess, A. F., and Myers, V. C.: J. A. M. A. **73**:1743, 1919.

in both instances entirely negative results and prompt excretion in the urine. Connor<sup>10</sup> injected the pigment dissolved in pure olive oil into the peritoneal cavity, producing by this method a small granuloma of foreign body type on the peritoneum and no absorption of the pigment.

Practically, however, all of the present knowledge of these pigments comes from the biologic chemists Kohl, Tsweet, Willstätter and his associates and Palmer and his co-workers.<sup>11</sup> They definitely proved the exogenous nature of the pigments even when the pigmentation occurs in the course of a metabolic disturbance. They also noted the lipochrome variation in the fat of different species of animals and birds even when they were fed on diets rich in carotinoids. On the same diet some are entirely free from pigment, while others have barely a trace, and still others have deeply colored fat. This must depend on some inherent characteristic, either an ability on the part of the bird to absorb carotinoids from the food and to deposit the pigment in the tissues or an ability of the bird to destroy the pigments and prevent their deposition. The normal absence of all traces of yellow pigment in some species speaks strongly against any real function of the carotinoids in life, a fact that receives strong corroboration from an experiment of Palmer's<sup>11</sup>: He was able to raise chicks to full maturity with normal fecundity on a diet entirely without carotinoids, producing eggs with colorless yolks which hatched, yielding healthy chicks.

A great variation in the amount of pigments absorbed occurs also among individuals of the same species and in experimental groups and in collections that, like the birds of this report, have been fed on a diet of the same carotinoid content and exposed to the same environment. With respect to the birds covered by this report, undue pigmentation was found in only forty-four of several thousand exhibited. The horse and the cow on a diet rich in carotin and especially rich in xanthophyll resorb carotin and exclude xanthophyll, although the latter is more abundant in the food. Fowls, on the contrary, exclude carotin and resorb xanthophyll. Mammalian bile has been studied and found to dissolve xanthophyll easily, but to be without effect on carotin. Whether this is a factor in the causes underlying the deposition of carotin is not known. There are no studies on avian bile. The bile from one penguin did not dissolve crystals of xanthophyll, but little weight can be given to one experiment. Gerould,<sup>12</sup> in a study of caterpillars, was able to increase the pigmentation by feeding an unusual amount of pigmented food. He thought that there was evidently a physiologic factor involved in these variations as between species, and that it probably was an enzyme, possibly an oxidase, free in the digestive tract. The individual variation is noticeably demonstrated in hens, a fact made use of by poultrymen in culling out unprofitable hens. Large production of eggs is definitely and positively correlated with pale shanks, ear lobes and beaks. In experimental forced feeding of xanthophyll the pigment appears in a very few days in the skin of nonlaying hens, but it may not appear for weeks in the skin or fat of laying hens, even if they lay only once or twice a week. In one feeding experiment two laying hens, one capon, and one sterile hen of the same breed, all apparently healthy, were fed on a rich carotinoid diet. The capon and the sterile hen colored intensely in five days, and at the end of four weeks the laying hens showed no appreciable change. Klose, in a study of infants from a home, stated that only a few of the babies on being fed carrots, showed the pigmentation, and these were always the best developed children, "all with well developed panniculus adiposus." Van den Berg and his associates in a study of the blood pigments of man found that the luteins were increased in diabetes, interstitial nephritis and arteriosclerosis, although pigmentation of the skin does not develop in all.

10. Connor, C. L.: *Am. J. Path.* **21**:227, 1928.

11. Palmer, L. S.: *Carotinoids and Related Pigments*, New York, The Chemical Catalogue Company, 1922.

12. Gerould, J. H.: *J. Exper. Zool.* **34**:385, 1921.

In the birds of this group milder and more severe lesions of the thyroid gland, pancreas, adrenal glands, kidneys and intestines were found at autopsy; all the birds had a tendency to accumulate fat. Apparently an excess of oxygen must be available to oxidize the pigments and so prevent their deposit in the tissues, and therefore any factor as caponizing, etc., that depresses the general metabolism or produces a suboxidation of the tissues favors the deposition of pigment.

PULMONARY. "PSEUDO-ACTINOMYCOSIS" (CHROMOBLASTOMYCOSIS?) IN A CAPYBARA (HYDROCHOERUS) DUE TO A HYPHOMYCETE PRODUCING RAY FUNGI IN THE TISSUE. FRED. D. WEIDMAN.

An unidentified fungus, which is not a member of the streptothrices (as is that in actinomycosis), but a broad-celled hyphomycete with thick walls, was established as the cause of a ray fungus formation in the lung of a capybara. The points to the presentation were as follows:

1. Radiate arrangements simulating ray fungus formation are possible in diseases other than actinomycosis; on passing inspection they could be readily confused with the ray fungus of actinomycosis.

2. Unique supplementary formations in the form of feathery lateral processes on the rays made their appearance.

3. As a broad hypha was demonstrable in the axis of some of the rays, the phenomenon is brought into analogy with ray formation in true actinomycosis, substituting only the broad, thick-walled hypha of a hyphomycete for the delicate, slender one of a streptothrix.

4. By analogy with other fungus cells that notoriously show incrustations (aspergilli, some of the microsporons, *Coccidioides immitis*), additional evidence is brought to bear on the debated nature of the hyaloid substance comprising the actinomycetic club; the fungus in this capybara serves as a welcome connecting link between the grosser, nonradiating forms just mentioned and *Actinomyces*. That is, the hyaloid constituents of the actinomycetic club are simply variant examples of the property of incrustation more or less general to higher fungi.

THE NATURE OF THE ERYTHROCYTIC DAMAGE PRODUCED BY ACETYLPHENYLHYDRAZINE IN VIVO AND IN VITRO. R. P. CUSTER.

Previous experimenters who have used acetylphenylhydrazine (pyroline) to produce anemia in animals have noted, on examination of stained smears of the blood, peculiar round, darkly-staining, refractile bodies, varying in size from one-eighth to one-sixth the size of the erythrocyte, lying either within, on or against the margins of the red blood cells. All have hazarded opinions as to the nature of these bodies and their relation to the hemolytic action of the drug. The work reported here is one phase of my experimental studies on phenylhydrazine compounds, showing the manner in which the erythrocytic structures are formed and thereby demonstrating the nature of the erythrocytic damage produced by these drugs.

Subcutaneous injection of acetylphenylhydrazine solution produces a profound anemia in forty-eight hours. The so-called "inclusion bodies" appear concomitantly with the anemia and persist until the regenerative phase of the blood is well established. Fresh preparations of the blood show the majority of the red blood cells to contain the bodies, definitely intracellular and marginal in position. The density of the body and the density of the cell vary inversely; the largest and most dense of the bodies are seen within "ghost cells."

A mixture of equal parts of acetylphenylhydrazine solution (1:100) with a 1:100 dilution of blood in isotonic saline solution, observed promptly in a hanging drop chamber, shows the mechanism of this reaction. A primary slight crenation of the erythrocyte is seen, followed within a minute by resumption of its original form, on establishment of osmotic equilibrium. Soon an opaque shadow appears within the cell, irregular and hazy in outline and occupying about one fourth of

the flat surface area. This shadow-like body becomes sharper of outline, more opaque and smaller (to about one eighth of the diameter of the cell); then increases in translucency and refractility, finally appearing as a vacuole. A given cell may contain one or more of these seeming vacuoles, which move about freely within the confines of the cell membrane. Further observation shows a slow fading of the hemoglobin of the cell, with a concurrent increase in the density of the contained body until but a shadowy cell membrane persists, immediately within the circumference of which lies the "inclusion body." Finally the cell membrane disintegrates, leaving the body free in the suspension. Spectroscopic examination of such a suspension gives the reaction for methemoglobin.

These bodies are analogous to the Heinz-Ehrlich bodies occasionally demonstrable in severe toxic anemias, the so-called "inner-body anemias."

This article is to be published in full in the *American Journal of Medical Sciences*.

A COMPARATIVE STUDY OF AMEBIASIS IN MAN, MONKEYS AND CATS, WITH SPECIAL REFERENCE TO THE FORMATION OF THE EARLY LESIONS. HERBERT L. RATCLIFFE.

A study of the histologic lesions of intestinal amebiasis in monkeys belonging to two South American genera has shown that the first lesions are superficial erosions of the mucosa. These are followed by the development of undermining ulcers that have their origin in the solitary lymph follicles of the wall of the large bowel. When tissue is fixed immediately after death, the amebas are seen in the mucosal lesions, but not in the submucosa. The apparent rôle of the amebas is the superficial destruction of the mucosa, which allows pyogenic bacteria to invade the lymph vessels and produce submucous abscesses in the follicles. The follicular abscesses rupture into the intestine and form the undermining ulcers.

Striking differences are seen when tissue is fixed from twelve to twenty-four hours post mortem. There are extensive necrosis of the mucosa and deep invasion of the wall of the gut by the protozoa. These organisms are also seen in large numbers in and about the ulcerated follicles. The inflammatory reaction about the ulcers, so prominent in fresh tissue, is not so striking. Leukocytes, as well as the remnants of follicles, undergo degeneration.

A comparison of these changes with the lesions described in human intestinal amebiasis shows that they are very similar and probably originate in much the same way. The presence of amebas deep in the tissues in man may be accounted for by postmortem invasion, since descriptions are made from tissue fixed several hours after death.

The lesions of intestinal amebiasis in the cat do not appear to be comparable to those in primates. In the cat, the pathologic process is one of direct invasion and destruction of the wall of the gut by the amebas. This is usually accompanied by an inflammatory reaction of the whole wall, indicating bacterial as well as amebic action. True undermining ulcers, comparable to those in primates, do not occur and lesions presented as such seem to have another explanation.

## Book Reviews

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THE REGULATION OF SIZE AS ILLUSTRATED IN UNICELLULAR ORGANISMS. By EDWARD F. ADOLPH, PH.D., Associate Professor of Physiology in the University of Rochester. Price, \$4.50. Pp. 233, with 66 figures and 15 tables. Springfield, Ill.: Charles C. Thomas, 1931.

This book is divided into twelve chapters: 1. Body Size as Structure and as Function. 2. Rates of Growth and Individuals. 3. Variability of Body Size. 4. Inheritance of Size. 5. The Rate of Multiplication. 6. Size in Relation to Multiplication Rate. 7. The Fusion of Individuals. 8. Internal Characteristics and Size. 9. Some External Factors and Size. 10. Size in Relation to Body Surface. 11. Size of Nucleus. 12. Conclusion. In addition, there are an appendix on spirogyra cells, an excellent bibliography and an index.

It is difficult to exaggerate the value of the quality and the quantity of data in this book. Also, merely to read the topics of a chapter is stimulating. In chapter 1, for example, a discussion of size as a regulative characteristic and of metabolic turn-over, one finds a treatment of regulation, of the cell theory, water as a constituent of cells, vacuoles, surface permeabilities, the lower limits of cells, etc. The chapter on rates of growth of individuals, dealing largely with *Paramecium*, shows the author's discrimination and critical judgment. Biologists will find the chapter on variability of body size the most useful. The old question of the relation of size to multiplication rate (chapter 6) is lucidly presented; so, too, are the chapters on internal characteristics and size and some external factors and size. The reviewer found the chapter on size in relation to body surface somewhat too briefly treated.

The book is a valuable contribution: (1) because of the large amount of carefully appraised data that it contains, (2) because of the clear and simple method of presentation and (3) because of its unified point of view.

Many readers, perhaps, may have some feeling of disappointment because Professor Adolph advances no theory. In this he but carries out the thesis expressed in the preface; namely, that the recognition and description of problems constitute the first step in the analysis and solution of these problems. One such problem is that of body size. He gives two main reasons for beginning the study of regulation of size on unicellular organisms: "First, their patterns and functions are most readily modified by changes of environment, so that influential factors may be quantitatively identified in isolation. Second, uniparental inheritance is the rule, with the result that, in any species, genetically uniform individuals may be obtained for study." These are two excellent reasons. The reviewer therefore agrees with Professor Adolph that physiologists have given too little attention to the advantage offered by unicellular organisms. Certainly, not since Verworn's time has the physiology of the protista received the attention it merits. The reasons for this situation are not difficult to ascertain.

Many biologists, including some protozoologists, consider unicellular organisms as apart from the multicellular. Also, many workers, including protozoologists, have somehow come to think of the protozoa too largely in terms of conjugation as exemplified by the ciliates. The emphasis placed on this one problem has therefore overshadowed problems of more general significance both for the protozoa and for the metazoa.

A general physiology, like Verworn's for example, based too much on protista, nowadays meets with a cool reception. On the other hand, a general physiology, in the sense of Claude Bernard, giving great emphasis to the vertebrate brain and spinal cord, finds ready approbation. However, the advantages of unicellular organisms pointed out by Professor Adolph cannot be gainsaid. Perhaps his book will stimulate new interest in the physiology of unicellular organisms. Certainly, it is a lucid statement which fulfils the promise to recognize and to describe the problem stated.

**DIE BIOLOGIE DER PERSON: EIN HANDBUCH DER ALLGEMEINEN UND SPEZIELLEN KONSTITUTIONSLEHRE.** VON PROF. DR. T. BRUGSCH UND PROF. DR. F. H. LEWY. Lieferungen 15 und 16, Band III. Pp. 577 and 888. Lieferung 17, Band II: Handschrift und Charakter. Von Elisabeth Flatow-Worms. Experimentelle Untersuchungen zur psychophysischen Typenforschung. Von F. H. Lewy, E. Jaensch, usw. Das Leib-Seele-Problem und die psychophysischen Korrelationen. Von Hans Pollnow. Paper. Pp. 695. Berlin: Urban & Schwarzenberg, 1930 and 1931.

With parts 15, 16 and 17, the "system of the biology of the person" has been completed. H. Haike discusses the relations between heredity, configuration of the external ear and diseases of the middle and internal ear. Much space has been devoted to otosclerosis, which among the hereditary diseases of the ear claims the greatest interest. Particular mention is made of the geneological trees of families with otosclerosis. Since it is impossible to predict otosclerosis with certainty, the author does not accept it as an indication for the artificial interruption of pregnancy. Valuable information will be found in F. Pinkus' splendid contribution on the skin. There are chapters on the finer structure of the skin, the features of the face, the secretion products of the skin, the effect of the physical influences of the outer world on the skin and its defense reactions, the relations between the endocrine glands and the skin, the action of poisons on the skin and the influence of diseases of the skin on body and mind. F. Munk, who is well known from his book on diseases of the kidney, takes up the normal and pathologic function of the kidney and its individual variations. A chapter has been devoted to the vegetative nervous system. K. Dressel and F. Himmelweit present the material in a clear and concise manner without going into details. The individual variations in the reactivity of the autonomic nervous system play an important rôle in modern clinical and experimental medicine. The autonomic nervous system is under continuously changing external and internal influences and, on the other hand, influences the normal and abnormal functions of the organs.

The last number gives an extensive contribution on hand writing and character by Elisabeth Flatow-Worms, which is profusely illustrated, and chapters on the experimental investigation into the psychophysis types (F. H. Lewy, E. Jaensch, W. Jaensch, O. Schmaehl, P. Lersch and C. Mandowsky) and on psychophysis correlations in general (H. Pollnow).

The whole work on the "biology of the person," the other issues of which have previously been reviewed, consists of four stately volumes. The material that has been collected in these volumes is enormous, and there are many chapters by physicians outstanding in particular fields of biologic and medical science. The fact that it contains data that are inaccessible to the average physician is perhaps the greatest value of the work. It brings attention to the tremendous importance of the problems of individuality in health and disease, which are still often neglected. Modern medicine has to deal not only with the causes and manifestations of diseases and their treatment, but also with the individual response to the morbid agent and to drugs. Constitutional pathology is the tenor of the work, which should be found in every well equipped medical and biologic library.

**RECENT ADVANCES IN FORENSIC MEDICINE.** By SYDNEY SMITH, M.D., M.R.C.P., D.P.H., Regius Professor of Forensic Medicine, University of Edinburgh, and JOHN GLAISTER, JR., M.D., D.Sc., J.P., Barrister-at-Law, Inner Temple, Professor of Forensic Medicine in the University of Egypt, Cairo. Cloth. Price, \$3.50. Pp. 194, with 66 illustrations. Philadelphia: P. Blakiston's Son & Company, 1931.

The object of this book is to review certain more or less recent advances in medicine in their medicolegal relations. The first four chapters are devoted to firearms and injuries by projectiles. The modern methods of identifying firearms receive thorough and competent consideration on the basis of much personal study.



All who deal with medicolegal problems of injuries from firearms will find these chapters of great value. The chapter on hairs is noteworthy because it describes and illustrates the microscopic appearances of the hairs of a large number of animals. The discussion of the medicolegal applications of the precipitin test and of blood grouping is elaborate but fails to convey that sense of experienced grasp of principles and practical details characteristic of the chapters on firearms and hairs. There follow short chapters on the quantitative determination of carbon monoxide in the blood, spectroscopy in medicolegal investigations, the detection of fluorescent substances by means of ultraviolet light and the estimation of alcohol in the blood and the urine. The illustrations are excellent. The book is commendable; it illustrates the great variety and refinement of methods that are applied in medicolegal work. Obviously, these methods are so diversified that no one person can master them all. Only organizations or institutes with several departments under expert direction can meet adequately the public needs in the way of scientific examinations for medicolegal purposes.

**OXIDATION-REDUCTION POTENTIALS.** By L. MICHAELIS. Translated from the German by Louis B. Flexner. (Monographs on Experimental Biology, founded by Jacques Loeb.) Price, \$3. Pp. 197. Philadelphia: J. B. Lippincott Company, 1930.

The discovery, by Hopkins, that all cells contain a substance, glutathione, capable of undergoing reversible oxidations and reductions has emphasized anew the importance of these reactions in the living cell. Oxidation-reduction (redox) systems give rise to measurable electric potentials that are dependent on the relative concentrations of oxidant and reductant in the system. The study of these electric potentials is thus of great importance in following the nature and course of such reactions.

The author considers the present volume as the second volume of his work on hydrogen ion concentration. The book is divided into two parts: Part 1, on physicochemical considerations, deals with the principles underlying the subject of oxidation-reduction potentials. Oxidation and reduction are first defined, in accordance with modern principles, as the loss and gain, respectively, of electrons. The author then discusses the thermodynamic basis for the calculation of electrode potentials and shows how this may be applied to a number of important inorganic and organic oxidation-reduction reactions. In the second part of the book, on physiologic applications, the author applies the principles developed and discussed in the first section to oxidation-reduction systems found in protoplasm. Such systems as the sulphhydryl (SH) systems, which are exemplified by cysteine and glutathione, as well as hemoglobin and its derivatives, cytochrome, etc., are discussed at great length. The purpose of this book is "to discuss the theoretical principles of oxidation reduction potentials" in order that future work in this important field might be facilitated. Professor Michaelis points out that "the present state of investigation forbids exhaustive discussion of the subject of physiological applications unless one be willing to risk the danger of involving immature topics."

**FOOD POISONING AND FOOD-BORNE INFECTION.** By EDWIN OAKES JORDAN, Chairman of the Department of Hygiene and Bacteriology, the University of Chicago. Price, \$2.50. Pp. 286. Chicago: University of Chicago Press, 1931.

This is the second edition of "Food Poisoning," published in 1917, which has been rewritten to include the results of later investigations. A brief enumeration of the contents will give an idea of the scope of the book. The first chapter deals with the general problem of food poisoning. Then come chapters on food allergy, poisonous animals and plants, poisons and harmful substances occasionally introduced into food by accident, and the deliberative addition of coloring matter or preservatives. There are two chapters on food-borne bacterial infections, with

special reference to paratyphoid food poisoning, and a chapter on animal parasites that may enter the blood in or on articles of food. The next chapter is devoted to the consideration of poisonous products developed in food by bacteria and other micro-organisms. In this chapter is discussed the toxic substance produced by staphylococci, which has been studied especially by the author and which probably causes food poisoning more frequently than is recognized. The final chapter deals with food poisonings of obscure or unknown nature. "There is an important residue of unexplained food poisoning that needs further skilled investigation. It is one of the objects of this book to point out this need and to draw attention to the numerous problems that await settlement. The first step is the regular and thorough investigation of every food-poisoning outbreak." The book is a model of clear and effective presentation. It summarizes exceedingly well the knowledge of the problems of food poisoning and its prevention.

## Books Received

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CATALOGUE OF THE NATIONAL COLLECTION OF TYPE CULTURES MAINTAINED BY THE COUNCIL AT THE LISTER INSTITUTE OF PREVENTIVE MEDICINE, CHELSEA BRIDGE ROAD, LONDON, S.W. Third edition. Medical Research Council, Special Report Series, no. 64. Price, 2 shillings net. Pp. 112. London: His Majesty's Stationery Office, 1931.

REPORT OF THE LABORATORY AND MUSEUM OF COMPARATIVE PATHOLOGY OF THE ZOOLOGICAL SOCIETY OF PHILADELPHIA IN CONJUNCTION WITH THE FIFTY-NINTH ANNUAL REPORT OF THE SOCIETY. Pp. 42. 1931.

STUDIES OF NUTRITION: THE PHYSIQUE AND HEALTH OF TWO AFRICAN TRIBES. By J. B. Orr and J. L. Gilks (for the Dietetics Committee of the Economic Advisory Council). Medical Research Council, Special Report Series, no. 155. Price, 2 shillings net. Pp. 82. London: His Majesty's Stationery Office, 1931.

A SYSTEM OF BACTERIOLOGY IN RELATION TO MEDICINE. Medical Research Council. Volume 8. Price, per volume, cloth, 1 pound, 1 shilling net. London: His Majesty's Stationery Office, 1931. (This may be obtained from the British Library of Information, 551 Fifth Avenue, New York.)

RESEARCHES IN BRITISH GUIANA 1926-1928 ON THE BACTERIAL COMPLICATIONS OF FILARIASIS AND THE ENDEMIC NEPHRITIS WITH A CHAPTER ON EPIDEMIC ABSCESS AND CELLULITIS IN ST. KITTS, BRITISH WEST INDIES. By A. W. Grace, M.B., and Feiga Berman Grace. Memoir Series of The London School of Hygiene and Tropical Medicine, no. 3. Pages 75. Price, cloth 10 shillings, 6 pence; paper, 8 shillings. London: London School of Hygiene and Tropical Medicine.

## ASSIMILATION OF ATLAS AND COMPRESSION OF MEDULLA

THE CLINICAL SIGNIFICANCE AND PATHOLOGY OF TORTICOLLIS AND  
LOCALIZED CHRONIC ARTHRITIS DEFORMANS OF THE SPINE

REPORT OF A CASE \*

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The upper part of the bone-walled channel that contains the medulla is solid. The lower part, on the contrary, is formed by separate bones—the atlas and the axis—and their articulations. As a consequence of the anatomic conditions, the channel may be narrowed, especially by upward or backward positional changes of the odontoid process of the second cervical vertebra, which forms part of the frontal wall. Under normal circumstances, these changes in the position of the odontoid process occur only by movement of the atlanto-occipital articulation. This movement, however, is extremely slight, as shown by the investigations of Virchow.<sup>1</sup> The movement is restricted by the resistance of the strong, tense ligaments around the articulations. Together with the special ligaments of the odontoid process itself, they assure the normal fixation of the odontoid process.

There are many pathologic changes that may alter the relation between the odontoid process and the medulla. This communication will deal only with changes in the bone wall of the channel, principally with the bones themselves and their articulations, although the latter are of less importance. These pathologic changes may be: total or partial destruction of the bones or interruption of their continuity. The occipital bone that forms the base of the skull, the atlas and the axis may be altered separately. But several members of this triad may undergo pathologic changes at the same time, and the articulations frequently participate in the process.

### REVIEW OF THE LITERATURE

In the literature of pathology, there are relatively few contributions to the knowledge of pathologic changes of the upper vertebral column

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1. Virchow, H.: Arch. f. Orthop. 26:1, 1928.

causing compression of the medulla. A review of these actual or possible changes, on the basis of general pathology, is therefore appropriate.

*Acute Suppurative Osteomyelitis.*—Acute suppurative osteomyelitis is limited in three fourths of all cases to a single vertebra. It is, as a rule, of metastatic origin and usually occurs in the second decade of life. It usually attacks the lumbar vertebrae and rarely those of the thoracic portion of the spine. In the case described by Donati,<sup>2</sup> it was localized in the second and third dorsal vertebrae, with symptoms of compression (peripachymeningitis). These symptoms ceased after operation, and the patient was cured. Simon's<sup>3</sup> case ended fatally in a few weeks, owing to compressive myelitis caused by extensive suppurative spondylitis. For some years the patient had chronic coxitis and spondylitis. In spite of this, Simon,<sup>3</sup> failing to detect any signs of tuberculosis, presumed that a cryptogenic septic infection had followed a fall that the patient had received. Recently, Berkheiser and Seidler<sup>4</sup> reported cases of acute arthritis of the atlas in children. In one case a roentgenogram showed a "strong forward displacement of the atlas—odontoid process near the posterior arch. . . . Nevertheless, no signs of compression were mentioned.

Of the inflammatory changes to be considered in this connection, the chronic inflammations are the more important. While tuberculosis is the most common of the chronic diseases of the vertebrae, other chronic inflammatory processes have been described.

Buka<sup>5</sup> reported a case of chronic suppurative panosteitis of the atlas, axis and third cervical vertebra caused by a staphylococcus. Purulent meningitis caused death. In Meier's<sup>6</sup> case, an extreme blocking of the foramen occipitale magnum was produced by luxation of the atlas caused by chronic arthritis deformans (polyarthritidis chronica deformans).

*Tuberculosis.*—Tuberculosis localizes most commonly in the lumbar column or in the lowest part of the dorsal column. It causes also caries of the cervical vertebrae. In a case of tuberculosis of the atlas, in which I performed the autopsy, the posterior arch and the lateral masses of the atlas were destroyed, and the injury involved all the joints of the atlas and axis. The atlas showed forward luxation, and the odontoid process had fallen backward and caused a sudden fatal compression of the medulla. Vertebral tuberculosis usually starts in the cartilages between two vertebrae. According to Brenner,<sup>7</sup> it is not uncommon in adults. Subsequent progression of the process in old, apparently healed, sclerotic lesions may make the anatomic diagnosis difficult at times. In

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2. Donati, M.: Arch. f. klin. Chir. **79**:1116, 1906.

3. Simon, A.: Deutschè Ztschr. f. Nervenhe. **32**:264, 1907.

4. Berkheiser, E. J., and Seidler, F.: J. A. M. A. **96**:517, 1931.

5. Buka, A. J.: Am. J. Surg. **8**:1280, 1930.

6. Meier, E. J.: Schweiz. Arch. f. Neurol. u. Psychiat. **24**:303, 1929.

7. Brenner, F.: Frankfurt. Ztschr. f. Path. **1**:293, 1907.

such a case, the differential diagnosis will be supported by the finding of tuberculous lesions in other organs, especially in the lungs, which are almost always affected in cases of vertebral tuberculosis. The pulmonary lesions may be old or of the same age as the vertebral lesions. On account of the clinical appearance and gross pathology, Stockert<sup>8</sup> regarded his interesting case of perforating tuberculosis of the base of the skull as one of malignant tumor. Histologic studies showed that the disease was tuberculosis. Clinically concealed tuberculosis often gives rise to spontaneous fracture.

*Syphilis*.—Syphilis seldom affects the vertebrae. Jasinski,<sup>9</sup> reviewing the literature, listed the following cases of this disease: (1) perispondylitis syphilitica of the cervical column, (2) osteitis gummosa of the first four vertebrae of the neck, (3) vertebral caries with gummas of the muscles, (4) infiltration around a kyphosis, gumma of the frontal bone and keratitis parenchymatosa and (5) caries gummosa of the clavicle and of the fifth and sixth cervical vertebrae. His diagnosis was supported by the efficacy of specific treatment. In the case of syphilitic caries of the second and third cervical vertebrae described by Zisché,<sup>10</sup> the bodies of these vertebrae were pushed out spontaneously through an ulcer of the posterior wall of the pharynx.

*Actinomycosis*.—The vertebrae of the neck are rarely injured by actinomycosis, although this disease, starting in the mouth, not infrequently occurs in this part of the body. Martens<sup>11</sup> described erosion and suppurative infiltration of one dorsal vertebra in a case of actinomycosis. This disease, arising probably in the lung, caused a chronic pleurisy with abscesses that broke through under the skin of the back.

*Echinococcus*.—Echinococcus may destroy parts of vertebrae. In the numerous cases of hydatid cysts of the vertebral column which have been recorded, the compressive myelitis was caused more often by the cysts themselves than by displacement of parts of the bones. These cysts occur most often in the lumbar or in the dorsal parts of the spinal column. The cysts are usually extradural and rarely develop within the substance of the medulla. In Souques'<sup>12</sup> case, the second lumbar vertebra was partially destroyed.

*Trauma*.—External trauma may cause fracture in the upper part of the cervical column, while luxation is usually inhibited by the resistance of the system of strong ligaments. It is noteworthy that compression

8. Stockert, W.: Beitr. z. Klin. d. Tuberk. **5**:507, 1906.

9. Jasinski, R.: Arch. f. Dermat. u. Syph. **23**:409, 1891.

10. Zisché, H.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **22**:357, 1911.

11. Martens: Arch. f. klin. Chir. **66**:698, 1902.

12. Souques, A.: Sitzungsab. d. anat. Gesellsch., Paris **10**:27, 1893; Centralbl. f. allg. Path. u. path. Anat. **5**:535, 1894.

of the medulla may be caused by the breaking off of the odontoid process as a result of some extraordinarily strong trauma. The case described by Dürck<sup>13</sup> is of especial interest in this connection. It throws light on both the pathogenesis of the condition and the extent of the alteration of the capacity of the canal necessary to produce compression of the medulla. An 18 year old girl, who had suffered a fracture of the odontoid process seven years previously while tobogganing, fell while dancing. Paralysis occurred rapidly and ended fatally the next day. Necropsy disclosed the old fracture of the odontoid process with false joints between the anterior arch of the atlas and the broken end of the odontoid process. There was contusion of the medulla, but no luxation of the atlas. There was secondary degeneration in the anterior and lateral pyramidal tracts of the lumbar spinal cord. The odontoid process broke off at its base, which is genetically its weakest part, where the "dens," belonging essentially to the atlas, will be connected by bone to the body of the second vertebra only at a later stage of development. The "dens," pushed backward, rose 7 mm. from the posterior edge of the ligamentum transversum into the vertebral channel. (Normally, according to Dürck,<sup>13</sup> the "dens" with the ligamentum transversum has a total height of 3 mm.) Dürck<sup>13</sup> considered this backward displacement a rare event. The broken odontoid process more frequently inclines forward, because the joint surface of the atlas tends to slide forward on the axis. In some cases, during life the odontoid process may become palpable through the posterior wall of the pharynx. In such conditions there is pain on swallowing. In later stages, the odontoid process may be thrown out, by suppuration, through the posterior pharyngeal wall. According to Bayard,<sup>14</sup> fracture of the body of the axis occurs much more rarely.

*Neoplasms.*—Both malignant and benign growths may narrow the bone-walled channel containing the medulla. According to Makrycostas,<sup>15</sup> hemangiomas developing within the vertebrae are not infrequent. These may so weaken the bone that traumatic or spontaneous fractures are easily produced. Tumors arising in the soft parts of the medulla itself will not be considered in this discussion.

#### AUTHOR'S CASE

An unusual case of assimilation of the atlas with compression of the medulla came under my observation in 1930. Its essential features were as follows:

13. Dürck, H.: Beitr. z. path. Anat. u. z. allg. Path. **84**:353, 1930.

14. Bayard, quoted by Dürck (footnote 13).

15. Makrycostas, K.: Virchows Arch. f. path. Anat. **265**:259, 1927.

*History.*—An assistant janitor, 33 years old, had been married six years and had one healthy child. During the war he was in the military service. After the war he was engaged in selling vegetables and eggs, bringing his supply in small quantities to Budapest. His two brothers are living and well. In 1927, during an attack of pleurisy, he had a thoracentesis. Until the onset of the symptoms of the condition to be described his wife had not noticed that he held his neck abnormally or crookedly. In 1928, his employer noticed that the patient did not turn his head toward a person who addressed him, but changed the position of his body by movements of his feet. During two years he complained of neck ache when lifting anything and often had severe headaches. Two weeks before his death, he held his head inclined to the right and could not turn his neck without difficulty. During these weeks he vomited frequently, and when attempting to move his head he felt pain in the suboccipital region. His gait became staggering like that of a drunkard, although he did not drink any alcoholic liquors.

On March 21, 1930, he was sent into the hospital by a physician, with a diagnosis of tuberculosis. At this time he complained of pain in the right side of the chest, dizziness, frequent vomiting, stomach ache and loss of appetite. He did not have fever.

On physical examination he was found to be well developed and fairly well nourished. The tongue and throat were clean, and the skin was free from any exanthem. The right side of the thorax was retracted. There was dulness on percussion, and there were diminished breath sounds over the back. On March 26, 1930, he died suddenly, without any remarkable previous symptoms or special examination. The clinical diagnosis was tuberculosis.

*Autopsy.*—The body was that of a well developed, fairly well nourished young man. There was extensive diffuse lividity of the dependent parts of the body. General rigor mortis was present. There were noted: a definite retraction of the anterior and upper part of the right side of the thorax, a somewhat tight cerebral dura and a somewhat increased amount of clear fluid between the pia and the arachnoidea. After the tentorium cerebelli was cut through and frontal lobes were drawn back, a plum-sized, transparent cyst appeared on each side of the medulla. Slight impressions on the cerebellum corresponded to these cysts. When the brain was removed, the cysts collapsed, but when the base of the brain was compressed they filled up again. The anterior edge of the cysts reached the seventh and eighth nerves; the posterior edge, the first cervical nerve. The cavities of the cysts lay beneath the arachnoid. At the bottom of the cysts, behind the flocculus, the choroidal plexus of the fourth ventricle was found. These cysts communicated with the fourth ventricle and were nothing more than large dilatations of the cisternae pontocerebellomedullares. On the anterior upper surface of the medulla, near the bridge, where the substance was definitely softer than the rest, there was a slight saddle-shaped impression, the size of a penny (fig. 1). The meninges around the chiasma were somewhat whitish. The fourth ventricle was enlarged, with considerable dilatation of the lateral recessus (fig. 2). A sagittal fissure 0.5 cm. long was found corresponding to the calamus scriptorius. The ependyma of the lateral ventricles seemed to be thickened around the blood vessels. The impressioes digitatae of the skull were very deep, and as a result of this, the bones, especially the squamae temporales, were very thin in places. The diploe of these bones were greatly diminished, and the bones were translucent. Near the inferior margin of the clivus, the dura showed an elevation, the size of half a walnut (fig. 3), which seemed on first inspection quite obviously to narrow the foramen magnum, especially in the sagittal diameter. The mass producing the elevation was immovable and hard, but felt somewhat elastic. After the dura on the upper



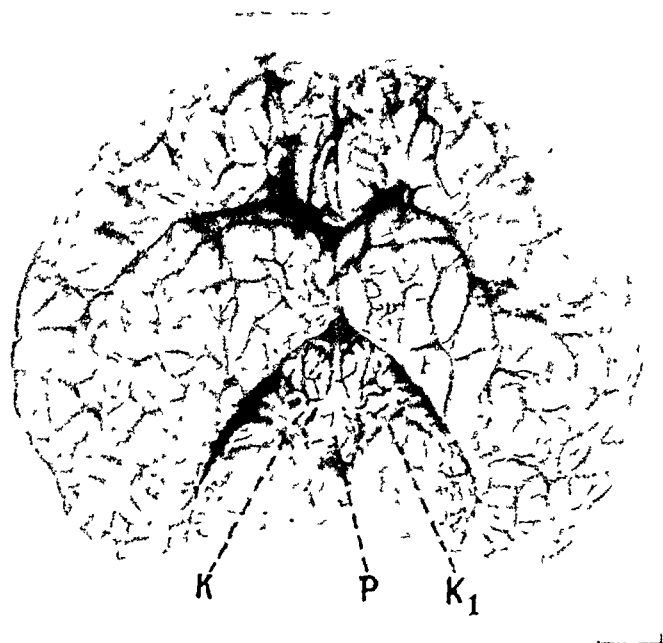


Fig. 1.—Base of brain: *P*, site of compression of medulla; *K* and *K*<sub>1</sub>, walls of arachnoidal cysts.



Fig. 2.—Dilatation of fourth ventricle: *S*, dilatation of calamus scriptorius.

margin of the elevation was cut through, it was found that the mass was not solidly connected with the clivus, but reached its posterior margin. The protuberance, narrowing the foramen magnum and compressing the medulla, could be identified as the enlarged odontoid process of the axis. On first impression, the enlargement was thought to be a new growth.

*Examination of Bones Around the Medulla.*—For the purpose of further examination the base of the skull and the first four cervical vertebrae were prepared in the following manner: One saw-cut was made through the base of the skull in a vertical plane at the tops of the petrous bones. An additional oblique cut on each side was made diagonally through the petrous bones from the external occipital protuberance. In this manner, the foramen magnum and the bones that form the upper part of the vertebral channel were preserved in a block.



Fig. 3.—Preparation "in block," view from above: *C*, clivus; *D*, protuberance narrowing foramen magnum; *M*, spinal cord; *H*, horizontal cut through membrana tectoria.

Before the deeper muscles of the neck or of the base of the skull were dissected, the whole specimen was hardened in 8 per cent formaldehyde. After fixation, roentgen pictures were taken.

The roentgenogram of the sagittal projection showed the bodies of three vertebrae (fig. 4). A triangular shadow of the external occipital protuberance fell across the lower part of the odontoid process, leaving the upper part of the process unobscured. The most striking feature was a bone shadow everywhere above the axis, and the outlines of the atlas could not be differentiated. The right jugular foramen and the right hypoglossal canal were clearly shown. The left hypoglossal canal was less distinct. The basilar part of the skull and the inferior part of the sphenoidal cavity were sharply distinguishable. Around the top of the odontoid process there was a fairly sharply limited, less dense bone

shadow, measuring from 2 to 3 mm., which became narrow on the left side. On the left, near the top of the odontoid process, there was a divided channel measuring from 2 to 3 mm. in diameter. Another extraordinary finding was the obliquity of the spinal column. The axes of the basal part of the occipital bone and of the vertebrae crossed. The former definitely inclined to the right. The third pathologic alteration was shown in the roentgen picture of the lateral view (fig. 5). Corresponding with the level of the supposed atlanto-epistrophic joint, the axis of the vertebrae bent forward, forming an obtuse angle of about 120 degrees, opening forward. The roentgenogram made after the removal of the

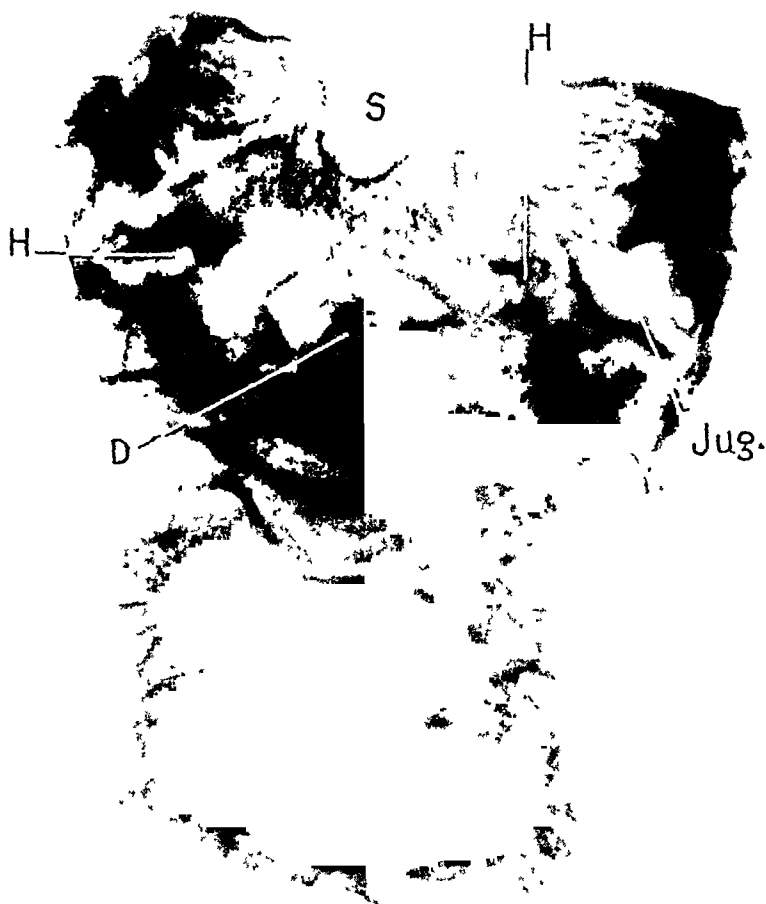


Fig. 4.—Roentgenogram, posterior view: *S*, sphenoid sinus; *H*, hypoglossal canal; *JUG.*, right foramen jugulare; *D*, odontoid process of axis.

arches of the vertebrae and a part of the back of the skull, with the posterior margin of the foramen magnum, was more valuable for the study of the conditions here. This picture definitely indicated absence of the atlas. The axis seemed to be joined directly to the skull. The surfaces of the upper articulations of the axis were different: the left was concave, while the right was irregularly convex. The comparison with the roentgenogram of a normal specimen (fig. 6) showed the hypoglossal canal of the pathologic specimen sunk almost to the level of the top of the odontoid process, while in the normal specimen there was a difference of 15 mm. between these points. The odontoid process was asymmetric.

Its right side, giving a more irregular and dense shadow than the normal, measured twice the length of the left side and inclined to the left. The process was a rounded cone shape, differing from the normal odontoid which narrows in the middle.

The roentgenograms, therefore, indicated absence of the atlas, or more correctly, of the atlanto-epistrophic articulation, and showed that the enlarged occipital bone was jointed directly to the axis. There were pathologic changes in this articulation. The roentgenograms showed, furthermore, that the vertebral column was inclined to the left—or, more correctly, that, with respect to the



Fig. 5.—Roentgenogram, lateral view: *E*, axis; *II* and *III*, cervical vertebrae; *O*, squama occipitalis.

vertebral column, the axis of the occipital bone was inclined to the right and forward.

Before dissection of the specimen, movability of the articulation in question was tested. Forward and backward flexion could be made easily and more extensively than in the normal specimen. Rotation, however, was nearly impossible, being much less than in the normal. Only a minimal change in position could be obtained by applying force in a direction imitating a turning of the head to the left.

The muscles and soft parts were removed for study, without previous maceration. They did not show any pathologic changes. The ligamentum

longitudinale anterior, corresponding with the atlas and axis, especially at the site of the capsule articulares, and the atlanto-occipital membranes seemed thicker than normal. They were easily removed. After their removal, the condition of the anterior surface of the preparation shown in figure 7 (right, normal specimen) was displayed. The occipital bone joined directly with the axis. The surface of this occipital bone was generally smooth, but on its lower side, parts of the atlas, such as the tuberculum anterior, and the two under-developed transverse processes could be recognized. The vertebral arteries were found in a normal position behind the latter. The divided channel, shown in the roentgenogram,

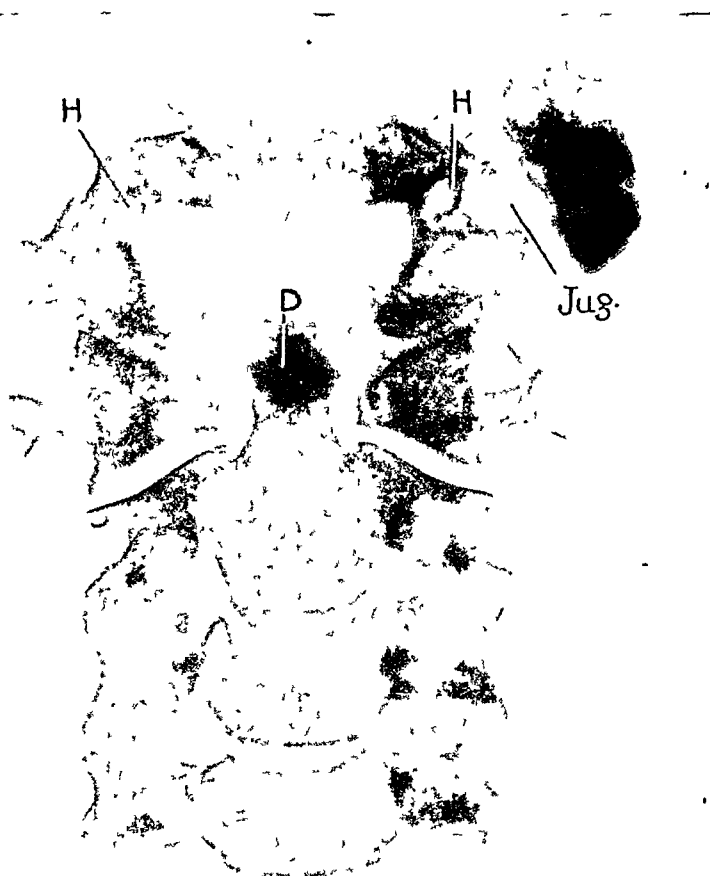


Fig 6.—Roentgenogram of normal preparation after the removal of arches of vertebrae and squama occipitalis, posterior view; *D*, odontoid process of axis; *H*, right hypoglossal canal; *Jug.*, right jugular foramen.

lay above and slightly to the left of the tuberculum anterior and contained small blood vessels and nerves. The hypoglossal nerves were seen above the origin of the small transverse processes. The right articular process of the os occipitale sprang forward as a roof, under which the upper right part of the axis was dragged (subluxation of the atlas!). Figure 8 showed that the axis of the three upper cervical vertebrae and that of the basilar part of the occipital bone in a line bisecting the sphenoid bone and passing through the tuberculum atlantis anterior crossed and formed an acute angle of from 10 to 15 degrees. In lateral

view, the axis of the vertebral column showed the deviation described. The vertex of the obtuse angle lay about at the base of the odontoid process. The spines of the first three vertebrae were to be seen to the right. The left arch of the axis covered the next arch behind like a roof. The left vertebral artery lay in the foramen transversarium of the axis. In posterior view (fig. 9; right, normal specimen), the posterior arch of the atlas was completely absent. Enlargement or thickening of the posterior margin of the foramen magnum could not be seen. The space between the occipital bone and the vertebra (*spatium epistropheo-occipitale posterius*) seemed to be narrower than that of the normal specimen (14 mm.). A motion corresponding to anterior flexion of the head greatly enlarged this space. The posterior arch of the axis projected backward 0.5 cm. over the posterior margin of the foramen magnum. The arch and spine of the axis were pushed to the left, and the left arch was thicker than the right. The cut end of the right artery lay in the foramen transversarium. After the removal

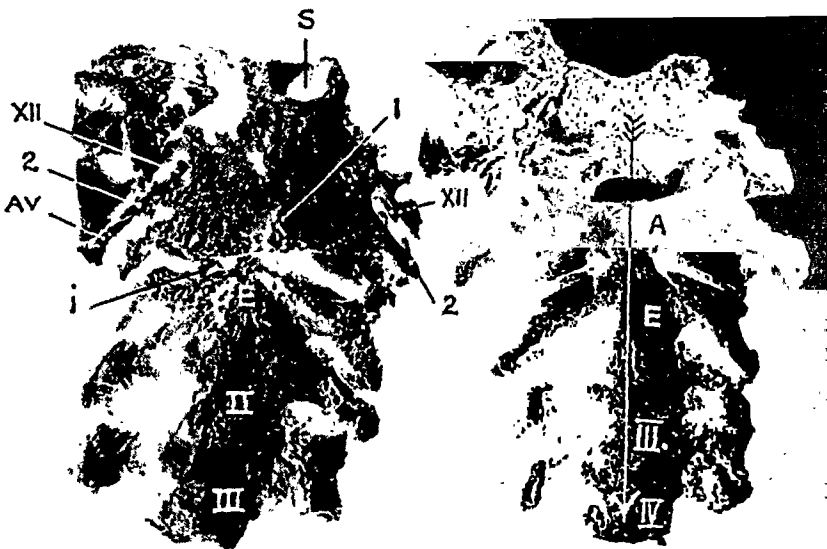


Fig. 7.—Anterior view of pathologic specimen (right, normal specimen): Periosteum almost removed; *I*, tuberculum atlantis anterior; *2*, rudimentary transverse process of atlas; *XII*, hypoglossal nerves; *i*, unevenness of joint surface; *A*, atlas; *E*, axis, *II-III-IV*, cervical vertebrae; occipito-axial joints opened; *S*, sphenoid sinus.

of small pieces of tissue from the posterior surface of the odontoid process and from a space on the right side showing ballotement, the cavities of the articulations were found. The roof of the latter articulation was represented by a layer of fibrous connective tissue, from 2 to 3 mm. thick, which contained a small amount of bone. On the lower, cartilage-covered surface of the odontoid process a small cavity of resorption was seen. In the view from above (fig. 10; right, normal specimen) after the arches of the vertebrae have been replaced, the clivus seemed to be flatter and wider than usual. The odontoid process completely filled the anterior dilatation of the foramen magnum. Its highest point, represented by structures supposed to be the ligamenta cruciatum and transversum, overlay the

lateral margins of the foramen magnum by from 5 to 8 mm. The anterior part of the top of the odontoid process was covered by a thin layer of fat tissue, and the narrow bone-walled fissure around the anterior circumference of the odontoid was similarly filled out by fat tissue. In this fat tissue a small vein and nerve coming through the small double channel were found. The vertebral arteries entered through pores located beside the odontoid process; the left was accompanied by the twelfth nerve; the right had a separate channel 2 cm. behind that of the twelfth nerve. Posteriorly, the uneven bottom of the joint cavity with two pea-sized elevations covered by cartilage was seen.

The situation of the deeper ligaments after removal of the membrana tectoria and ligamentum longitudinale posterius is shown in figure 11 (right, normal



Fig. 8.—Anterior view of pathologic preparation after removal of muscles and superficial ligaments. An arrow indicates axis of vertebral column; an interrupted line, that of os basale and sphenoid. Note subluxation of right inferior articular process of occipital bone. Left joint has been opened.

preparation). The odontoid process had only one ligament, which was inserted in both lateral parts of the anterior dilatation of the foramen magnum. This ligament covered the odontoid process like a cap and served as the posterior capsule of the joint. It was partially adherent on both sides, measured from 1 to 1.5 mm. in thickness and evidently corresponded to the ligamenta cruciatum and transversum of the atlas. (The normal system of ligaments of the atlas and axis are seen in the control specimen; they are ligamenta cruciatum, ligamenta alare, etc.).

*Examination of Articulations of the Head.*—There was only one articular cavity, since both occipito-axial articulations were connected in their lateral and



Fig. 9.—Posterior view of pathologic specimen (right, normal preparation): *B*, cartilaginous joint surface of odontoid process; *J*, opened superior joint cavity of axis; other marks as in figure 7.



Fig. 10.—View of pathologic specimen from above (right, normal preparation): Periosteum partially removed; *C*, clivus; *D*, odontoid process; *U*, unevenness of joint surface. Note flattened shape of clivus in pathologic preparation.



posterior parts with the posterior joint cavity of the odontoid process. On the joint surfaces there were large defects in the cartilage. Some smaller areas covered with cartilage were irregularly elevated, like protuberances. The surfaces of the upper articulations of the axis were much enlarged, but were only partially covered by cartilage. The defects of cartilage were most conspicuous in the posterior parts. In this area pea-sized cartilaginous islands were seen (figs. 10 and 11). Both of these articulations were saddle-shaped, with rooflike, irregular elevations in their midfrontal areas. The elevation on the right was larger than that on the left. The right joint surface showed near its anteromedial margin a bony protuberance with a somewhat uneven surface, the size of a small pea (fig. 7). This protuberance coincided with an adequate ditch of the "occipital" joint surface, deprived of cartilage. The immobilization of the head might have been due chiefly to this pathologic connection of both joint surfaces. The articulations of the occipital bone, also, had pea-sized defects of cartilage on the lateral



Fig. 11.—Posterior view of pathologic preparation after removal of arches of vertebrae (right, normal preparation): Deeper ligaments of odontoid process are shown; *D*, ligamentum cruciatum; *AL*, ligamentum alare; *C*, clivus; *A*, atlas; *E*, axis; *II-III-IV*, cervical vertebrae.

parts of both surfaces. The whole left surface was somewhat smoother than the right. The anterior and lateral part of the odontoid process also seemed to be covered by cartilage. Nevertheless, it was partially rough, and adherent, especially on both sides, to the ligamentum cruciatum. The anterior side had a pepper-sized prominence of cartilage in place of the normal joint surface. On the right side of this, an irregular ditch of similar size indicated adhesion with the atlas part of the occipital bone. Thus only the anterior side and the top of the odontoid process failed to be entirely covered by cartilage. To substitute for the destroyed cartilage of the articulation there were tongue-like or meniscus-like soft processes of the thickened articular capsule, which ended freely in the articular cavity. The largest tongue-shaped process covered a large ulcerated area on the posterior margin of the right superior axial articulation.

The right inferior articulation of the axis was represented by only a pea-sized cartilaginous spot. There was scarcely any cavity here. The left inferior articulation of the axis, as well as the articulations of the other vertebrae, were entirely normal.

The changes in the size of the foramen occipitale magnum are shown in the accompanying table of measurements. As a consequence of its penetration into the cavity of the spinal foramen, the odontoid process produced changes in three directions, namely upward, backward and somewhat to the left. Measurements of the normal specimen are given for comparison.

*Histologic Examination.*—Specimens were taken from the ligament of the odontoid process, from the fibrous articular capsule on the right side of the odontoid process (see fig. 9), from several parts of the periosteum and joint capsules and from the membrana "axo-occipitalis" posterior. Tissue was taken also from the second and third segments of the cervical portion of the spinal cord, from the right lung and from the right pleura.

The ligamentum cruciatum showed hyaline connective tissue, with few cells, and in some places resembled cartilage. The articular surface was almost devoid

*Size of the Pathologic Foramen Magnum in This Case Compared with the Normal*

	Pathologic	Normal
Difference in height between the apex of the odontoid process and the incisura marginalis posterior foraminis magni.....	+20 mm.	+ 2 mm.*
Difference in height between the apex of the odontoid process and the incisura marginalis anterior .....	0	— 8 mm.
Difference in height between the apex of the odontoid process and the incisura marginalis lateralis .....	+ 4 mm.	—12 mm.†
Distance between the posterior surface of the cruciform ligament and the incisura marginalis posterior .....	19 mm.	29 mm.

\* When the head is tipped back the posterior margin of the foramen magnum falls from 1 to 2 mm. below the horizontal line.

† This measurement was not taken in a true perpendicular plane, but somewhat toward the direction of the lateral odontoid ligament.

of endothelial cells. In material removed from the capsule of the right occipito-axial joint next to the odontoid process, there were particles of real bone containing bone marrow in the midst of hyaline connective tissue. Specimens from various parts of the capsules, as well as from the periosteum, revealed hyaline connective tissue with few cells. The tongue-like, meniscus-shaped structures described contained fat tissue and were mostly covered by endothelial cells.

The central canal of the cervical portion of the spinal cord was greatly dilated and had long, bowed, fissure-like processes covered by flattened ependyma, extending into both posterior horns (fig. 12). In the central halves of the posterior bundles there was extensive destruction of the fatty sheaths, with large accumulations of granulated cells. This destruction was especially obvious in Clarke's column, but it was also apparent in the adjoining parts of the gray substance.

The pleura of the right lung showed fibrosis, with occasional small blood vessels and capillaries surrounded by an infiltration by round cells. In the substance of the lung, the interstitial connective tissue was somewhat increased, and the alveoli were partially or totally compressed. There was an accumulation of polymorphonuclear leukocytes within the bronchi.

*Pathologico-Anatomic Diagnosis.*—The pathologico-anatomic diagnosis was: assimilation of the atlas; relative dislocation of the odontoid process backward

and upward; narrowing of the foramen magnum due to this dislocation; peculiar form of arthritis deformans in the articulations between the first vertebra (axis) and the skull; colliquation of the anterior part of the medulla by pressure of the odontoid process; dilatation of the fourth ventricle of the brain and of subarachnoidal cysts, approximately 1 by 2.5 cm., on each side of the medulla, communicating with the recessus lateralis of this ventricle; moderate chronic basilar leptomeningitis; thinning of the bones of the skull with deep impressioes digitatae; hydromyelocele below the level of the compression of the medulla; destruction of myelin sheaths with accumulation of granular cells in the Goll-Burdach bundles and Clarke's column; chronic fibrous pleurisy, on the right, with retraction of the chest and partial atelectasis of the lung; purulent bronchitis; extreme congestion of all the internal organs, especially of the liver, spleen and kidneys; moderate distention of the urinary bladder; thick, dark red blood.

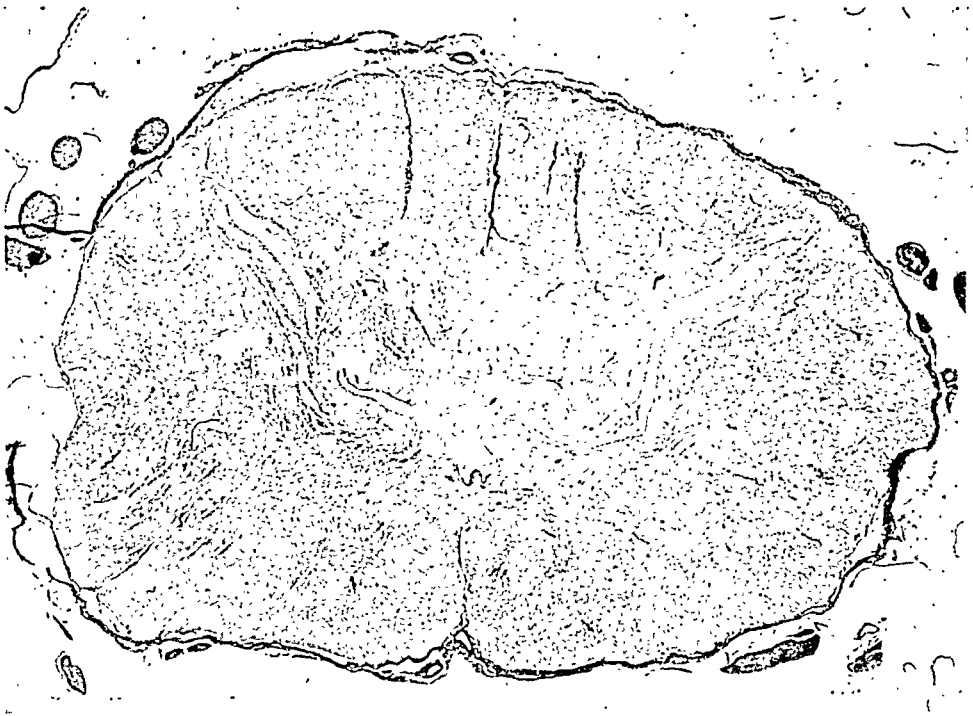


Fig. 12.—Section of second segment of cervical part of spinal cord: Dilatation of central canal (hydromyelocele) with long bowed processes into both posterior horns covered by flattened ependyma.

#### COMMENT

In this case the cause of the sudden death was the compression of the medulla. This diagnosis is supported by the observations at autopsy and by the results of histologic examination. The sudden death may also be explained by the hydrocephalus internus, which actually seemed to be circumscribed, being localized mainly in the fourth ventricle. The subarachnoidal cysts might also have caused pressure on the cerebellum and medulla. The grade of the hydrocephalus probably fluctuated frequently, and one has to assume that it had suddenly increased just before death. The fluctuation of the hydrocephalus and the development of

pathologic hydrostatic conditions might have been caused by the progressing disease, producing a gradual constriction about the medulla. The irregular thinning of the bones of the skull is to be considered as a result of increased pressure within the cranial cavity for a very long time. The finding is of great importance in its bearing on the possibility of establishing an acceptable explanation of the origin of the disease.

The degeneration of the myelin sheaths found in the posterior column and in the gray matter of the spinal cord was the result of a long-standing narrowing process or of a compression of the cervical spinal cord by hydromyelocele. The hydromyelocele was due also to the compression of the medulla. It is well known that disturbances of the circulation of the spinal fluid are important in the pathogenesis of hydromyelocele. The latter may develop in the spinal cord above or below the compression. The degeneration of the myelin sheaths about Clarke's column and the posterior cornua might have been produced by the pressure of the hydromyelocele.

The compression of the medulla, therefore, developed slowly as the result of a narrowing process of the foramen magnum. The degree of narrowing corresponds to the observation of Nonne.<sup>16</sup> In his case ("malum suboccipitale") tuberculosis had destroyed the joints of the atlas and axis. A chronic dislocation developed between the atlas and the axis and between the base of the skull and the atlas. The atlas fused with the base of the skull, and the axis with the atlas. The atlas slid forward and the odontoid process of the axis penetrated into the foramen magnum. The posterior rim of the odontoid process, owing to the dislocation, came 1 cm. nearer to the posterior arch of the atlas. The same dislocations occurred in this case. In Nonne's case the upward dislocation of the odontoid process was 2.5 cm.; in this case it was 1.8 cm. In Dürck's<sup>13</sup> case the backward dislocation was smaller than in this case—the fractured and backward dislocated odontoid process together with the transverse ligament protruded 7 mm. toward the spinal canal. Dürck added that this distance is only a small fraction of the sagittal diameter of the spinal canal. However, it is enough to cause a mild pressure on the dura and spinal cord.

The medulla extends from the upper border of the atlas to the midportion of the clivus and here lies between the jugular tubercles. It is approximately 2.5 cm. in length, its breadth measures from 10 to 11 mm. below, from 17 to 18 mm. above, and from 9 to 15 mm. in the anteroposterior dimension. On the narrowing of the bony channel about the lower end of the medulla in Nonne's case and in this case, there remained a space 19 mm. in diameter for the medulla and other soft tissues. In Dürck's case an even larger space remained (22 mm.).

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16. Nonne, M.: Arch. f. Psychiat. **74**:264, 1925.

Here the medulla measured 10 mm. in diameter. In my opinion, the possibility of compression in a given case is dependent not only on the thickness of the contained soft tissues, but also on the amount of the cerebrospinal fluid accumulated about the medulla. In this case, one has to consider also the effect of an increased internal pressure on the spinal cord caused by the hydromyelocele. Although the examination has not been completed, the fact that the hydromyelocele extended as far as the medulla was shown by a depression in the calamus scriptorius, the latter being a connection between the fourth ventricle and the central canal. In this case the two fixed and probably constant points, between which the compression occurred, were formed by the upper posterior surface of the odontoid process and the posterior rim of the foramen magnum. In the cases described by Nonne and Dürck, however, the posterior arch of the atlas must also be taken into account, although other prominences on the canal wall might have caused pressure at different points as the position of the head changed. In this case the compression was increased when the subarachnoidal cysts on both sides of the medulla were completely filled.

*Classification of the Conditions of the Bones Involved.*—It is much more difficult to determine the development of the pathologic condition than to determine the primary cause of death. In order to do that, several questions have to be answered besides classifying the pathologic changes: What is the significance, first, of the pathologic change of the atlas, and second, of that of the occipito-axial joint? Furthermore, what is the chronological relationship between these two pathologic conditions? Were they caused by the same factor, or did they develop separately, from different causes? Finally, is there any possibility of a causal relationship between the two pathologic conditions?

A pathologic union between the atlas and the axis has not infrequently been found. Lombroso<sup>17</sup> found synostosis of the atlas in 0.84 per cent of the soldiers fallen in the battle of Solferino, while Legge<sup>18</sup> found the same pathologic condition in 0.64 per cent of 780 cases (cited by Sommer<sup>19</sup>). Monteiro and Tavares<sup>20</sup> reported 0.08 per cent occipitalizations among 1,176 craniums. According to newer knowledge this synostosis may be congenital or acquired—the latter being usually the result of inflammatory processes. In such instances certain deformations occur on the vertebrae, that is, exostoses, irregular thickenings or bony ridges. In other cases, the atlas may grow together with the skull and may form a part of the skull without the presence of previous pathologic

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17. Lombroso, C., quoted by Sommer (footnote 19).

18. Legge, F.: Arch. di psichiat. **4**:384, 1883.

19. Sommer, W.: Virchows Arch. f. path. Anat. **119**:362, 1890.

20. Monteiro, H., and Tavares, A.: Compt. rend. Soc. de biol. **99**:960, 1928.

processes. Kollmann<sup>21</sup> called this union an assimilation, on the basis of two of his own cases. In his first case the arch of the atlas was developed, the posterior was thinned out and opened to a distance of 1 cm. The ventral atlanto-occipitale space became a narrow passage as the result of the assimilation. In his second case the assimilation was more marked; a considerable part of the posterior arch was absent. Hayek<sup>22</sup> found assimilation of the atlas in human embryos and new-born infants, and in animals. In a case of assimilation the axis does not lose its characteristics; it is not transformed into an atlas. The observation was also made that the third vertebra is not transformed into an axis. In such cases the occipital bone has no occipital condyles, but it shows an inferior articular facet similar to that of the atlas.

In this case a congenital, therefore true, assimilation of the atlas occurred. This opinion is based on the facts that the rudiments of the atlas were very well outlined on the anterior surface of the wall of the foramen magnum, that the vertebral arteries had a regular course and that the posterior arch of the atlas was completely absent. That the thickening of the left arch and spine of the axis was due to the downward growth of the atlas cannot be considered. By this determination one could rule out inflammatory processes and other pathologic changes thought of in connection with the question of etiology. Histologic examination spoke against pathologic lesions of specific inflammatory or other nature. Autopsy did not show any signs of tuberculosis. As I have previously mentioned, even in healed Pott's disease a tuberculous focus of similar duration must be present somewhere in the body. By ruling out inflammation and by establishing the congenital origin of the assimilation of the atlas I also have been able to trace a definite chronological order in the course of the deformities found on these bones and joints. It would seem reasonable to apply the name "assimilation" of the atlas only to congenital cases, while for all others the name "synostosis" should be used.

A synostosis may interfere with a cysternal puncture, as was pointed out by Anton<sup>23</sup> and others. This is very unlikely in the case of assimilation of the atlas, because under such circumstances there is, as a rule, an absence of the posterior arch of the atlas, thus facilitating puncture. It is a fact, however, that by tipping back the head in this case the posterior space between the occipital bone and the axis was made smaller than the posterior atlanto-occipital space of the control specimen.

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21. Kollmann, J.: *Verhandl. d. anat. Gesellsch.* **19**:231, 1905; *Anat. Anz.* **30**:545, 1907.

22. Hayek, H.: *Jahrb. f. Morphol. u. mikr. Anat.* **58**:269, 1927.

23. Anton, G.: *Deutsche Ztschr. f. Nervenhe.* **89**:2, 1926.

On the other hand, if by roentgenogram and puncture an occlusion of the posterior space is detected, according to our present knowledge the condition is, most probably, a synostosis, therefore an acquired condition and not an assimilation.

Another pathologically important feature of a union of the atlas with the occipital bone was pointed out by Anton.<sup>23</sup> He found nine cases of epilepsy among thirty-one persons showing "eine Verwachsung oder ganz nahe Kontinuität des Atlas mit dem Hinterhauptbeine am Profilröntgenbilde." In one case, autopsy, it was said, proved the roentgen observations. It must be mentioned that Förster, who was present, did not find the pathologic change demonstrated by Anton.

Bertolotti and Mattiolo<sup>24</sup> reported the occurrence of "congenital cranio-vertebral malformation" in three cases of complex Friedreich-P. Marie disease. The x-ray picture in the first case showed, according to the authors, atlo-axial occipitalization and vertical median segmentation of the posterior arch of the atlas and furthermore partial axo-assimilation of the third cervical vertebra.

Therefore, the observations made to date do not seem to have proved definitely the pathologic importance of the assimilation of the atlas. I agree with Hayek<sup>22</sup> in this opinion.

*Conditions of the Joint.*—The deformities of the joint corresponded in part to those found in chronic arthritis deformans, although they showed some essential differences from these. Chronic arthritis deformans may develop from an acute purulent arthritis, or it may develop in an essentially chronic manner. In this case, although there were no supporting clinical data, one may assume that the empyema that was noted three years previously might have caused purulent inflammation of this joint by metastasis. Purulent arthritis is usually not superficial, but often extends to the outer layers of the membrane of the joint and to the periarticular tissues. In the latter instance, an abscess develops about the joint. There is frequently a pathologic involvement of the cartilage of the joint in cases of purulent arthritis, viz., a necrosis of the cells, preceded by fatty degeneration and splitting of the matrix into fibers. After the immigration of leukocytes, the cartilage becomes liquid or separated from the bone. There develops a sequestering or rarefying osteitis in the denuded bone, resulting when completely healed, in ankylosis. If the cartilage does not degenerate, as occurs in a milder case after the inflammatory process has subsided, the capsule of the joint shrivels up, and the result is limited motion (pseudo-ankylosis). The purulent inflammation may become chronic. At such times, the inflammation stops on the development of a bone-forming osteitis fibrosa on the denuded joint surfaces, provided death does not ensue or provided

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24. Bertolotti, M., and Mattiolo, G.: *Chir. d. org. di movimento* 6:253, 1922.

the soft tissues are not involved. Thus the denuded joint surfaces grow together (true ankylosis). The synovial membrane becomes fibrosed and during the granulation the suppuration ceases.

In this case neither pseudoankylosis nor true ankylosis was present. The cavities of the occipito-axial joint were rather wider than normal. The synovial membrane did not show inflammatory thickening. Histologically it was loose and rich in blood supply, and in many places an endothelial lining could be demonstrated. I think that a metastatic arthritis originating from the empyemia that was present three years previously may be ruled out on the basis of these findings.

Three forms of essentially chronic arthritis are distinguished: The first, ulcerous arthritis sicca is a disease of old age. Frequently it develops in one joint, especially that of the hip; therefore the name "malum coxae senile." It is not an inflammation, but a disturbance in the nutrition of cartilage and bone. The cartilage cells undergo fatty degeneration; the matrix is split into fibers; finally it falls apart, and the edge or the whole surface of the cartilage becomes ulcerated. The denuded bone is transformed into a partly porous, partly sclerotic, substance. A thickening of the synovial membrane occurs, without, however, new formation of bone. The second form of chronic arthritis, adhesive arthritis (chronic rheumatism) is a disease involving numerous joints. Its distribution is similar to that of acute "rheumatism" (acute polyarthritis), and it develops occasionally on the basis of this disease. As a rule, however, its onset is insidious from the beginning; it gradually gets worse until the end of life, and in a severe case destroys all the joints of the body. The characteristic pathologic changes are: an inflammatory thickening of the capsule of the joint and degeneration of the cartilage, which subsequently is replaced by connective tissue. The synovial membrane is transformed into a hard, fibrous connective tissue, often with the fibrous capsule, ligaments and tendons. The union of the capsules obliterates the recesses of the cavity of the joint. There is no exudate, the synovia being of an unusually small amount. The degeneration and ulceration are very similar to that occurring in arthritis sicca, but in contrast with the latter, the degenerated cartilage is replaced by connective tissue, which regenerates from the synovial membrane. These connective tissues later result in the union of the joint surfaces (fibrous ankylosis). Bony tissue may also be formed in this connective tissue (osseous ankylosis). Subdislocations of joints of hands and feet may occur, with clubbed position; the corresponding muscles become atrophic. A third form, chronic arthritis deformans, is characterized by degeneration and atrophy, and on the other hand by a regeneration of cartilage and bone. The areas showing much lack of material correspond to those subjected to the highest pressure. In the areas of the degenerated cartilage the bone becomes sclerotic (through fibrous osteomye-



litis) and even may become ivory-like. In either case these areas are polished smooth through motion. The rough surfaces of the opposing bones make furrows in each other, which are parallel in the joints with one axis. Independently of this polishing, circumscribed cavities and widespread defects develop as a result of subchondral lacunar resorption. The head of the joint becomes flat. Simultaneously with the destruction of the surface of the joint a widening of the same takes place by bulging of the rim of the cartilage (peripheral ecchondrosis—Pommer's "Randwülste"), in an older case the bulging being caused by a periosteal exostosis. Accordingly, the rim of the joint shows similarities to a mushroom. The capsule of the joint usually presents an inflammatory thickening, especially at its reflection. The thickening is due to enlargement of the villi of the joint. The villi grow out to long threads, and they may cover the whole surface, or they may be found only on the rim of the joint or in the recess of the reflection (villose joint, "Zottengelenk"). The hypertrophied villi are commonly made up of connective tissue ("papillary fibroma"), although they may contain fat tissue, or even may be formed exclusively by fat tissue ("lipoma arborescens"). Villi of the latter type are thicker and more bulky than the "fibromas." During movements they break off easily and fall into the cavity of the joint. Bone may also be formed in the villi and be torn off, resulting in a so-called "joint mouse." Neither exudate nor union belong to the picture of chronic arthritis deformans. According to Pommer,<sup>25</sup> the important pathologic indication of this disease is the ingrowth of a marrow-containing bone to the rim of the joint cartilage, thus giving rise to a so-called "Randwulst." Chronic arthritis deformans occasionally involves numerous joints. In the majority of cases, however, only one, especially the hip joint, is affected. This monarticular form is often caused by injuries or by excessive use of the joint in certain vocations. It may also follow some other inflammatory processes, possibly tuberculosis, and in the latter instance occurs in childhood.

The etiology and pathogenesis of chronic arthritis deformans is not fully understood. According to the functional theory of Pommer<sup>25</sup> and Benecke,<sup>26</sup> diminished elasticity of the cartilage is the beginning change. Schmidt<sup>27</sup> also believed that the cartilage is the seat of the primary change. Lang's<sup>28</sup> opinion was that the greatest importance regarding the etiology and pathogenesis should be laid on the mechanical

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25. Pommer, G.: *Virchows Arch. f. path. Anat.* **219**:261, 1915.

26. Benecke, R.: *Fortschr. a. d. Geb. d. Röntgenstrahlen* **33**:843, 1925.

27. Schmidt, M. B.: *Handb. d. path. Anat.* **2**:240, 1928; *Centralbl. f. allg. Path. u. path. Anat.* **41**:24, 1928.

28. Lang, F. J.: *Med. Welt* **15**:557, 1928.

and functional injuries affecting the joints. Wagner<sup>29</sup> pointed out the significance of muscular activity. Disturbances of the latter have an important influence on the development of the pathologic conditions of the joints. In addition, chronic internal injuries of the joint may also act as causes. Heine<sup>30</sup> did not accept the functional-mechanical theory of Pommer and Benecke or the statical theory of Preiser.<sup>31</sup> Heine, after various considerations regarding the etiology, attributed much, though not the greatest, significance to humoral effects, these being in close relationship to heredity, constitution and disposition. Consequently, one meets with widely varying, rather obscure causes in the etiology, as well as in the pathogenesis. Axhausen,<sup>32</sup> contrary to Pommer, pointed out the importance of a primary necrosis of the cartilage in the development of chronic arthritis deformans. Heine<sup>30</sup> admitted, however, that in certain conditions abnormal positions of the joint may secondarily induce arthritis deformans and that the former may have great influence on the course of a primary degenerative arthritis. Therefore, Heine attributed only a secondary importance to the functional-mechanical theory.

In comparing the pathologic changes in this case with those discussed in the preceding paragraphs, it becomes evident that the condition here cannot be classed with the first two forms of chronic arthritis. It was similar to chronic arthritis deformans to the extent that the joint surfaces were enlarged, and that there was more or less lack of cartilage. Consequently, the joint surface became very rough, though there was an absence of the typical "Randwulst" that occur in classic arthritis deformans. The cartilage-covered elevations were not found on the rim of the joint, but rather toward the center. They were comparatively low and probably were due to a degenerative disappearance of the adjacent cartilage. The behavior of the synovial membrane of the capsule was also different; instead of "lipoma arborescens," peculiar menisci were developed exactly filling out the spaces of the rim that were deficient in cartilage. In order to save the specimen, a histologic examination of the joints was not carried out. On the basis of the described findings, however, I classify the condition as belonging to chronic arthritis deformans. According to all findings, the changes developed secondarily; thus one is dealing with a secondary arthritis. I expect to analyze and discuss the peculiar deformities of the joints in this case in the future. A succinct explanation of their development may be given as follows: The odontoid process of the axis abnormally approached the

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29. Wagner, K.: *Med. Klin.* **23**:1258, 1927.

30. Heine, J.: *Virchows Arch. f. path. Anat.* **260**:521, 1926.

31. Preiser, G.: *Deutsche Ztschr. f. Chir.* **89**:540 and 613, 1907.

32. Axhausen, G.: *Berl. klin. Wchnschr.* **52**:1205, 1915.

medulla because of the assimilation and rudimentary development of the mass of the atlas. It caused a narrowing of the bony canal from birth. This narrowing caused, on the one hand, a disturbance of the circulation of the spinal fluid, increasing intracranial pressure, and, on the other hand, compression of the medulla. It is probable that the latter circumstance provoked the abnormal position of the head, in consequence of which the mechanism of the joints of the head was changed, resulting in abnormal function. The abnormal function—lack of function, specifically—during a period of time caused both the partial destruction of the cartilage of the joints and the atrophy of the bony end-ports. Simultaneously with the progressive changes in the joints there gradually developed a diminution of space about the medulla, thus producing a vicious circle. Accordingly, in this case the chronic arthritis deformans was due purely to a pathologic change of function—specifically, to mechanical-static conditions. Therefore, one may explain the etiology of the arthritic condition in this case by the functional theory.

Of the small joints, the right inferior joint surface of the axis was diminished, as was the contiguous joint surface of the adjacent "second" vertebra. The diminished surface might have been caused by the dislocation and was probably related to the forward subdislocation of the right "occipital" joint surface. In the formation of this joint deformity a deviation of the articular processes might have taken a part, or there may have been a retardation in the development of this joint.

The question of why the disease developed only in the later years of life, causing indirectly the death of a 33 year old person, may be chiefly answered by a disposition of age. One must also consider changes in the circumstances of life (the indigences of war and overwork). Heine<sup>20</sup> pointed out the importance of heredity, constitution and disposition in the obscure etiology of chronic arthritis deformans. In this case, however, the constitutional factor, the malformation of the bone, seems to have been the most important.

Another question requiring an answer is: What is the explanation of the fact that pathologic changes similar to these have not been found. In the reported cases (Kollmann<sup>21</sup> and others) anatomic specimens were presented, which had previously been macerated, and the past history of which was probably unknown. But even these cases differ from this one in the degree of assimilation. In the case of Kollmann, there was only a partial assimilation, and the bony substance was not hindered in development. It seems, therefore, that the important factor is a considerable degree of assimilation of the atlas and a defective development of the bony mass which may result in compression of the medulla. Furthermore, it is also possible that similar cases remained obscure because of the difficulty of examination and because of lack of reports of similar observations.

## SUMMARY

Among the pathologic changes of bones which may cause compression of the medulla, the dislocation of the odontoid process of the axis seems to be the most important. Observations show that this dislocation is mostly caused by partial destruction of the bones around the medulla due to inflammation or to trauma.

According to my observation, the odontoid process may become "dislocated" in consequence of an assimilation of the atlas, and may cause compression of the medulla. But it seems that the assimilation must reach a certain degree, while the mass of the atlas remains underdeveloped. This assimilation of the atlas causes pathologic changes similar to those in arthritis chronica deformans, owing to the abnormal mechanical-statical conditions. This, therefore, supports the functional theory of the etiology of arthritis chronica deformans. The pathologic function has to be regarded as secondary i. e., as a consequence of the primary constitutional factor—the malformation of the atlas.

As to the compression of the medulla, the alterations of the articulations described are of great importance. By their successive development, the volume of the bone-walled channel around the medulla is changed accordingly, and in this way a vicious circle arises between compression and the conditions in the joint.

On the basis of this case, and according to the cases published, a lethal compression of the medulla may occur when the odontoid process is dislocated at least from 7 to 10 mm. backward, and from 18 to 25 mm. upward. In considering compression, the development of hydrocephalus and hydromyelocele is of great importance.

As the atlas may be more or less melted together with the os occipitale after various inflammatory processes, I would propose that the term "assimilation" be reserved for congenital cases, i. e., those based on malformation. The adhesions of the bones arising in extra-uterine life (in most cases with inflammatory origin) should be called "synostosis."

Only synostosis may become a hindrance to puncture of the cisterna. It is probable that in the case of assimilation, in which, as a rule, the posterior arch of the atlas is absent to a greater or less extent, puncture of the cisterna is easier.

## CONCLUSION

From the results of the examination of this case evidence has been obtained demonstrating the pathologic importance of assimilation of the atlas and supporting the functional theory of the etiology of a peculiar form of arthritis chronica deformans.

# EXPERIMENTAL RICKETS AND CALCIFICATION OF DENTIN \*

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By detailed research, McCollum and Simmonds,<sup>1</sup> Shipley and Park,<sup>2</sup> and Sherman and Pappenheimer,<sup>3</sup> as well as others, have extended knowledge of experimental rickets in rats. In the course of their nutritional studies on the formation of bone they succeeded in proving that the ratio of calcium to phosphorus, within certain limits, is of greater importance than the absolute amounts. If the ratio is ideal, i. e., a relative excess neither of the one nor of the other, a comparatively small amount of vitamin D will insure normal skeletal development. On the other hand, if there is a relative excess of phosphorus over calcium, or vice versa, small amounts of vitamin D cannot prevent severe disturbances of the bones.

However, by compensating for the relative lack of calcium or phosphorus or by increasing the amount of vitamin D in the diet, normal formation of bone could be induced. In other words, vitamin D is able to compensate, in part at least, for the disproportion of the mineral salts. Abnormal bone is formed when these three factors are altered, and the typical picture of true human rickets is produced when there is an improper ratio of calcium to phosphorus in the absence of vitamin D. Many articles have been written describing the several diets that will produce these severe changes in the bones, and of especial

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1. McCollum, E. V.; Simmonds, N.; Shipley, P. G., and Park, E. A.: *J. Biol. Chem.* **47**:507, 1921; **51**:41, 1922; *Am. J. Hyg.* **1**:492, 1921; **2**:97, 1922. McCollum, E. V.; Simmonds, N.; Becker, J. E., and Shipley, P. G.: *J. Biol. Chem.* **53**:293, 1922. McCollum, E. V.; Simmonds, N.; Kinney, E. M., and Grieves, C. J.: *Bull. Johns Hopkins Hosp.* **33**:202, 1922.

2. Shipley, P. G.; McCollum, E. V., and Simmonds, N.: *J. Biol. Chem.* **49**:399, 1921. Shipley, P. G.; Park, E. A.; McCollum, E. V., and Simmonds, N.: *Dental Cosmos* **3**:265, 1922.

3. Sherman, H. C., and Pappenheimer, A. M.: *Proc. Soc. Exper. Biol. & Med.* **18**:193, 1920-1921; *J. Exper. Med.* **34**:189, 1921.

interest are McCollum's diets 3127, 3133, and 3143. These are set forth in the table.

Diet 3127 is deficient in phosphorus and is practically free from vitamins A and D. It contains adequate protein, and the amount of calcium is close to the optimum. When young rats are fed on this diet, they grow for a short time, depending on the amount of vitamin A stored in their livers; then severe ophthalmia and rickets develop and the rats die early.

A small change in the diet, the addition of a little butter fat, is sufficient to improve the curve of growth and to retard the development of the disease of the eyes. This occurs with diet 3133; it produces a more characteristic picture of rickets, as the animals live longer.

Diet 3143 resulted from a study to find the relation of phosphorus to rickets. There is sufficient vitamin A to prevent sore eyes and to

*McCollum's Diets for the Production of Severe Changes in the Bones*

Diet 3127		Diet 3133		Diet 3143	
Rolled oats*.....	40.0	Rolled oats*.....	40.0	Wheat†.....	33.0
Gelatin.....	10.0	Gelatin.....	10.0	Maize (yellow).....	33.0
Wheat gluten.....	7.0	Wheat gluten.....	7.0	Gelatin.....	15.0
Sodium chloride.....	1.0	Sodium chloride.....	1.0	Wheat gluten.....	15.0
Potassium chloride.....	1.0	Calcium carbonate.....	2.0	Sodium chloride.....	1.0
Calcium carbonate.....	2.0	Potassium chloride.....	1.0	Calcium carbonate.....	3.0
Dextrin.....	39.0	Dextrin.....	38.5		
		Butter fat.....	0.5		

\* Finely ground.

† Wheat and yellow corn finely ground.

induce fair growth; the diet is low in phosphorus and high in calcium and has sufficient vitamin B and sufficient vitamin G to prevent beriberi and pellagra. This diet contains no vitamin D. It produces enough growth to make a wide metaphysis free from calcification, and rachitic lesions best suited to histologic study.

According to Shipley and Park,<sup>2</sup> young rats fed diet 3143 show some changes in their movement after from eighteen to twenty days, when kept in a dark or a semidark room. They no longer walk in straight lines, but stagger from side to side. Frequently weakness of the hind legs develops that may lead eventually to definite paralysis. The first signs of impaired movement are simultaneous with characteristic changes in the bones.

Shipley and Park used for their microscopic examinations the distal end of the femur and the proximal end of the tibia. In normal bones the epiphysis is separated from the diaphysis by a distinct straight line. In rickets this demarcation is ragged and may be of diagnostic value. Diet 3143, the "line test diet" produces a typical line, which is free from calcification and thus can be used to test substances for their vitamin D content. Shipley and Park showed that the nature of the changes

induced is not constant, and that the x-ray picture cannot give the desired information; therefore a histologic study of the long bones is essential.

Three types of changes are produced in the growing skeleton: (1) typical rickets, (2) atypical rickets and (3) osteoporosis.

Typical experimental rickets presents the microscopic picture of human rickets; that is, osteoid margins, increased depth and length of the epiphyseal cartilage, irregular vascularization in the cartilage and metaplasia of the latter to osteoid tissue. The rachitic zone between the cartilage and the bone develops in the same manner as in human beings.

Atypical rickets differs in that the changes are not so pronounced, and occasionally there is partial calcification, while heavy osteoid margins are found on the bone trabeculae. Sometimes the picture of osteoporosis is combined with that of rickets.

Shipley and Park in their histologic examination of rachitic material did not describe the effects on the jaw bones and teeth. A description of macroscopic conditions of the teeth and mouth has been given by Grieves.<sup>4</sup> This did not permit an analysis of the histologic changes in either the teeth or the paradentium of these rats.

Since a tooth arises from the epithelial and mesenchymal tissues (enamel and dentin), it would seem to be an ideal medium for the study of the calcification and development of hard substances. Dr. E. V. McCollum has put the "fixed" skulls of a large series of these experimental rats at our disposal, enabling us to complete the histologic picture of experimental rickets and to describe the involvement of the teeth and of the surrounding tissues.

#### MATERIAL

The upper and lower jaws of twenty-one rats were examined. All these animals had been on the line test diet, 3143, for from seven to forty-two days. Their growth was fairly satisfactory, the animals being about 25 days old when first placed on the diet. In order to study the sequence of the effects of the diet on the development of the teeth, groups of three animals each were examined, which had been seven, fourteen, twenty-one, twenty-seven, twenty-eight, thirty-five and forty-two days on the diet.

#### HISTOLOGIC EXAMINATION

The histologic examination revealed distinct changes in the dentin, which increased during the course of the experiment. The photomicrographs reproduced here are all of the same magnification to facilitate orientation (except figs. 7, 12, 16 and 17). They represent typical

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4. Grieves, C. J.: The Effect of Defective Diets on Teeth, J. A. M. A. **79**: 1567, 1922.

stages of dentinal development as found in the incisors of each group. The molar teeth could not be used as well for this examination, as they are not of continuous growth and had almost completed their growth at the time the experiment began. There were, however, several items of note in the molar area as described later.



Fig. 1.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for seven days: *a*, normally calcified dentinal matrix; *b*, zone of irregular globular calcification; *c*, demarcation line; *d*, calcoglobuli; *e*, predentin; *O*, odontoblastic layer; *E*, enamel; *U E E*, united enamel epithelium. The specimen (1205 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 150:1.



The incisors were cut lengthwise in such a manner that central longitudinal sections from the crown through the apical foramen were available for examination. The photomicrographs were taken at the same distance from the apical foramen and on the buccal surface of the



Fig. 2.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for fourteen days: *a*, normally calcified dentinal matrix; *b*, zone of irregular globular calcification; *d*, calcoglobuli; *e*, predentin; *x*, group of atrophic odontoblasts; *y*, group of vital, well-stained odontoblasts; *E*, enamel, fallen out in decalcification; *U E E*, united enamel epithelium. The specimen (1217 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 150:1.

tooth in order to compare the changes more accurately. Serial sections were cut from all blocks.

Because of the normal nutrition during the first twenty-five days, a zone of uniformly calcified dentin is visible histologically in all animals. This layer has been denoted as *a* in all figures.



Fig. 3.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-one days: *x*, group of atrophic odontoblasts; *y*, deeper stained, vital odontoblasts, with corresponding continued calcification. The specimen (1222 B) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4  $\times$ ; extension of camera, 80 cm.; reduced from a magnification of 150:1.

After seven days (fig. 1) it can be seen that another fairly well calcified dentinal layer, *b*, exists in contact with the normal zone *a*. There is a definite line, *c*, between these two zones. Layer *b* is not straight as under normal conditions, due to an irregular, globular calcification,

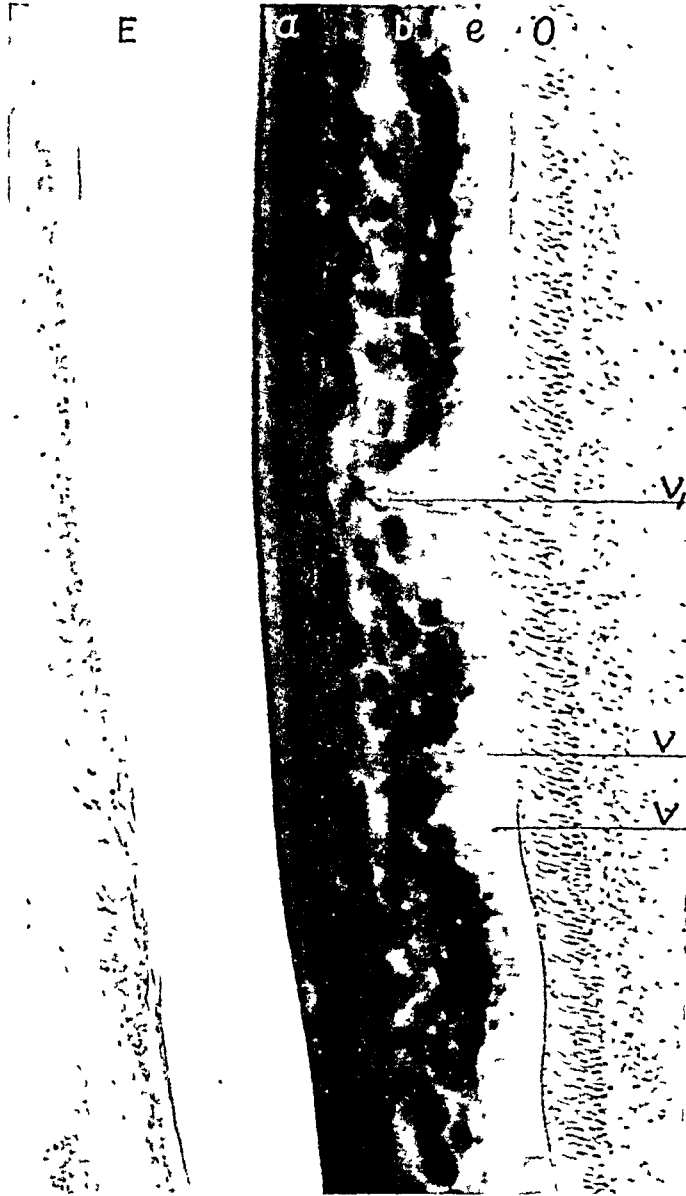


Fig. 4.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-seven days: *a*, normally calcified dentinal matrix; *b*, zone of irregular globular calcification; *c*, predentin; *v*, included blood vessels; *v*, included blood vessel, with obliterated distal end; *O*, odontoblasts; *E*, enamel. The specimen (1234 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 150:1

*d.* The predentinal layer, *c*, is abnormally wide. The odontoblasts are in normal arrangement and seem to be of equal vitality.

After fourteen days (fig. 2), it can be recognized that the predentinal layer is a great deal wider. The irregularly calcified zone, *b*,

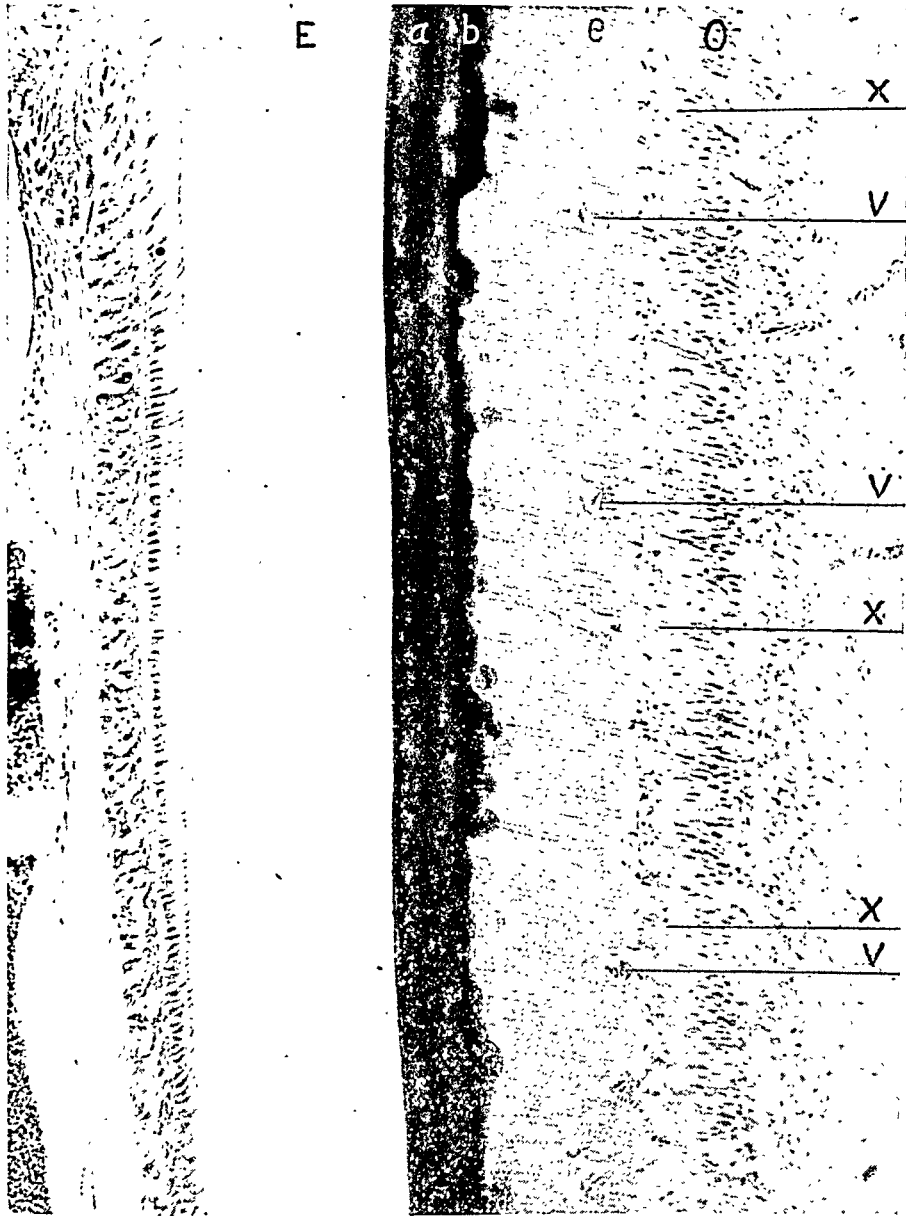


Fig. 5.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-seven days: *a*, normally calcified dentinal matrix; *b*, zone of irregular, globular calcification; *c*, predentin; *x*, advanced areas of degeneration in odontoblastic layer; *v*, included capillaries; *O*, odontoblasts; *E*, enamel. The specimen (1234 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 150:1.

is more pronounced. The odontoblastic layer is well stained, except for several definite areas, as at *x*. The odontoblasts in this area seem to be in a state of complete exhaustion; this might explain the stopping of the calcification in the adjacent dentinal matrix. These odontoblasts are in contrast with the deeply stained ones at *y*, with a corresponding continuation of calcification. The widening of the predentinal layer suggests that the matrix has continued to form at the normal rate, but that the calcification has slowed down.

Figure 3 apparently shows the same conditions further advanced after twenty-one days. The predentinal layer is about the same width, but involvement of the odontoblastic layer is greater. Areas of well stained odontoblasts alternate with others poorly stained; and corresponding to these areas are, alternately, zones where calcification has continued and others where it has been stopped for some time.

Figures 4 and 5 are taken from the same specimen, from a rat that had been for twenty-seven days on the diet. Figure 4 shows an area incisal to the typical position. The latter is shown in figure 5. Virtually the same disturbances in calcification are shown in both pictures. It can be observed that the zone of irregular globular calcification in figure 4 is considerably wider and the predentinal layer much narrower than in figure 5. Blood corpuscles are plainly seen in the included vessels found in the predentin of both areas. This indicates that they were active at necropsy. These capillaries, which can be traced directly from the pulp, are found in greater numbers as one advances toward the apex. The vessel  $v_1$ , in figure 4, is obliterated at its distal end, which probably accounts for the calcification in close proximity to that portion of it.

In figure 5 the odontoblasts are seen to be in a more advanced stage of degeneration than those shown in figures 2 and 3. This degeneration is uniform in figure 4, whereas in figure 5 it has affected small alternate groups. Adjacent to these most advanced areas of degeneration an apparent ingrowing of capillaries is observed. This was first described by Erdheim.<sup>5</sup>

Marked disturbance in the formation of the dentin matrix is found after twenty-eight days (fig. 6). Many capillaries are included in the matrix. A definite border is no longer found at the edge of the predentin. As a result the odontoblasts have a wavy, irregular appearance instead of their usual parallel arrangement, and show definite pathologic changes. An almost complete loss of staining power is noticed, and they can hardly be differentiated from other cells of the pulp. An

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5. Erdheim, J.: *Frankfurt. Ztschr. f. Path.* **7**:175, 1911; *Denkschr. d. k. Akad. der Wissensch., Math.-naturw. Klasse*, 1914, vol. 90; *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1906, vol. 16.

increased number of vessels run into the predentin in an irregular manner, and are there inclosed by the ground substance. It seems as if the degeneration of most of the odontoblasts had started early, and that only a few have continued active. For instance, at *t* (fig. 6), several places are seen where the formation of ground substance has

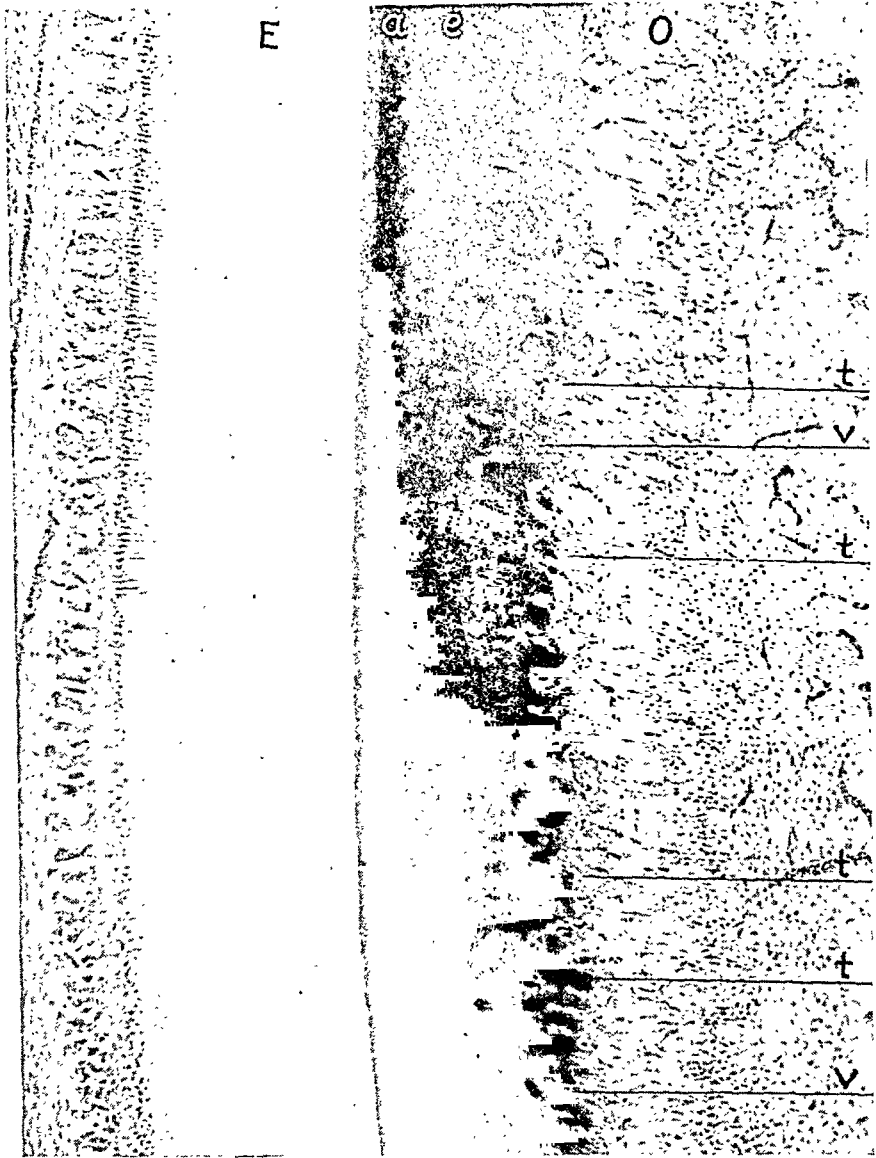


Fig. 6.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-eight days: *a*, normally calcified dentinal matrix; *c*, predentin; *t*, areas of prolonged formation of ground substance and Tomes' fibrils; *v*, included capillaries; *O*, layer of odontoblasts, greatly disorganized; *E*, enamel. The specimen (1258 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 150:1.

continued, with distinct formation of Tomes' <sup>6</sup> fibrils. Adjacent to these areas the inclusion of capillaries may be noted, probably because no ground substance was formed.

Figures 7, 8 and 9 show that these changes are not always produced, even under similar nutritional conditions. These photomicrographs are

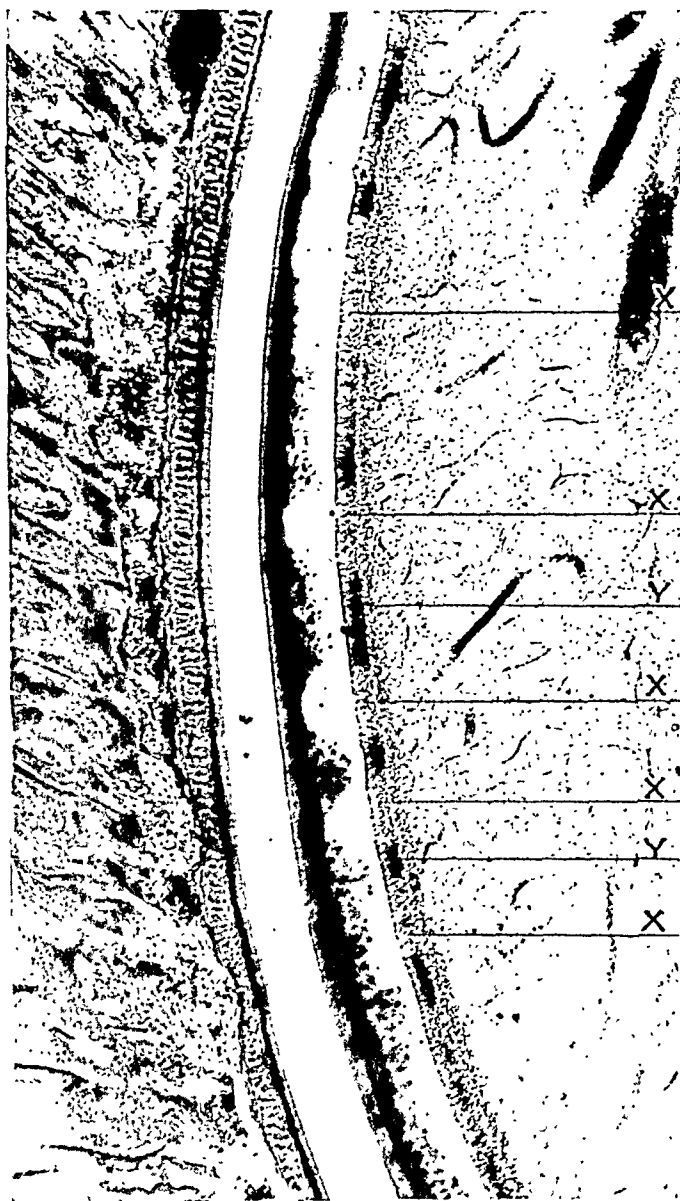


Fig. 7.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-eight days: *x*, group of atrophic odontoblasts; *y*, group of vital, well stained odontoblasts. The specimen (1261 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective, 1 b; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 55:1.

6. Tomes, Charles: *Deutsche Vrtljschr. f. Zahnh.*, 1878, no. 2, p. 159.

taken from animals that were also on the rachitic diet for twenty-eight days. Included vessels are not found in the predentin. In these specimens the intermittent degeneration of the odontoblasts, *x*, with a corresponding cessation of calcification, is conspicuous. In some places where the odontoblasts are still vital, *y*, and stain intensely, globular calcification has continued.



Fig. 8.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-eight days: *x*, group of atrophic odontoblasts and cessation of calcification; *y*, group of vital odontoblasts and continued calcification. The specimen (1261 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 150:1.



The pathologic changes of the odontoblastic layer in this animal have not progressed so far as in the animal of figure 6, although both were on the diet for twenty-eight days.

Figure 10 shows an area of hypoplasia of enamel. This was seen only in animals that had been on diet 3143 for at least twenty-eight

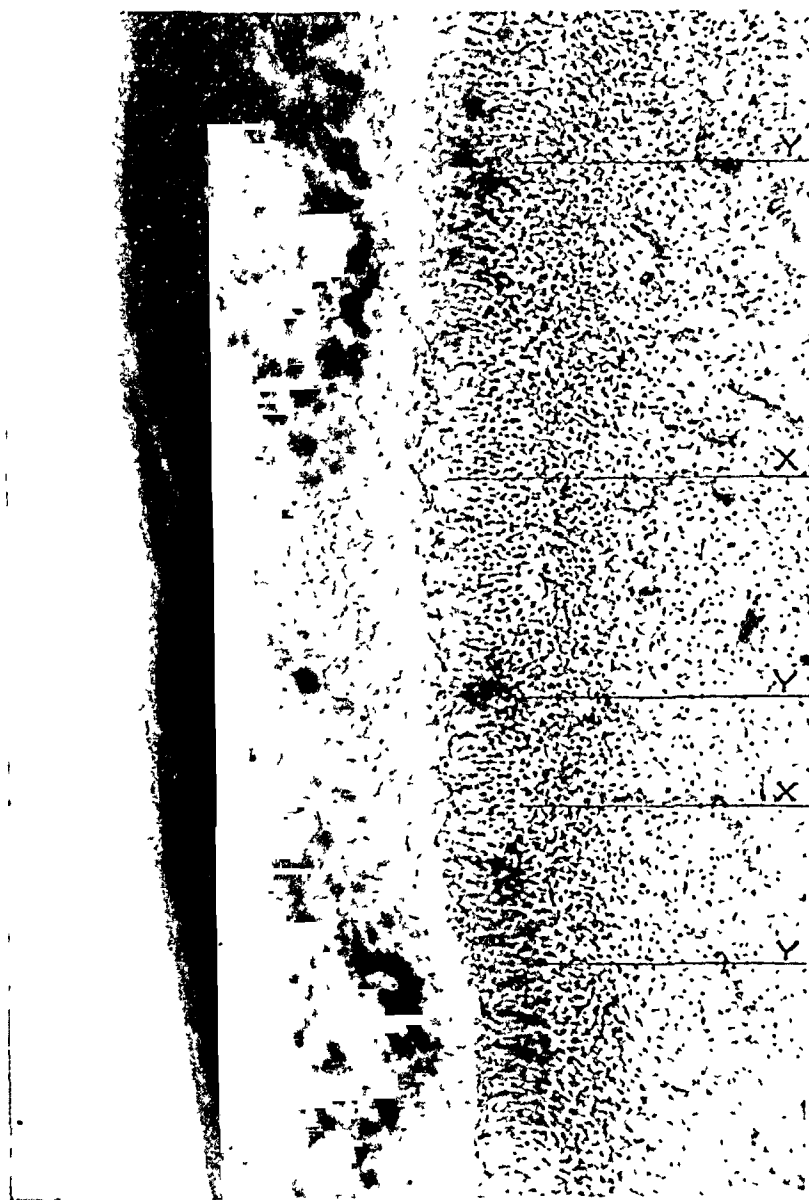


Fig. 9.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-eight days: *x*, group of atrophic odontoblasts; *y*, group of vital odontoblasts. The specimen (1262 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 X; extension of camera, 80 cm.; reduced from a magnification of 150:1.

days. Ameloblasts in their typical form are not visible in the hypoplastic area. More detailed description of this condition will be given in a subsequent report.

The changes after thirty-five days of the diet are not essentially different from those after twenty-eight days. As may be seen in figure 11, the involvement of the odontoblasts and the consequent cessation of calcification in the predentin are present.

Figure 12 is near the incisal edge; figure 13 is a higher magnification of the same area, and figures 14 and 15 are closer to the apex of the same

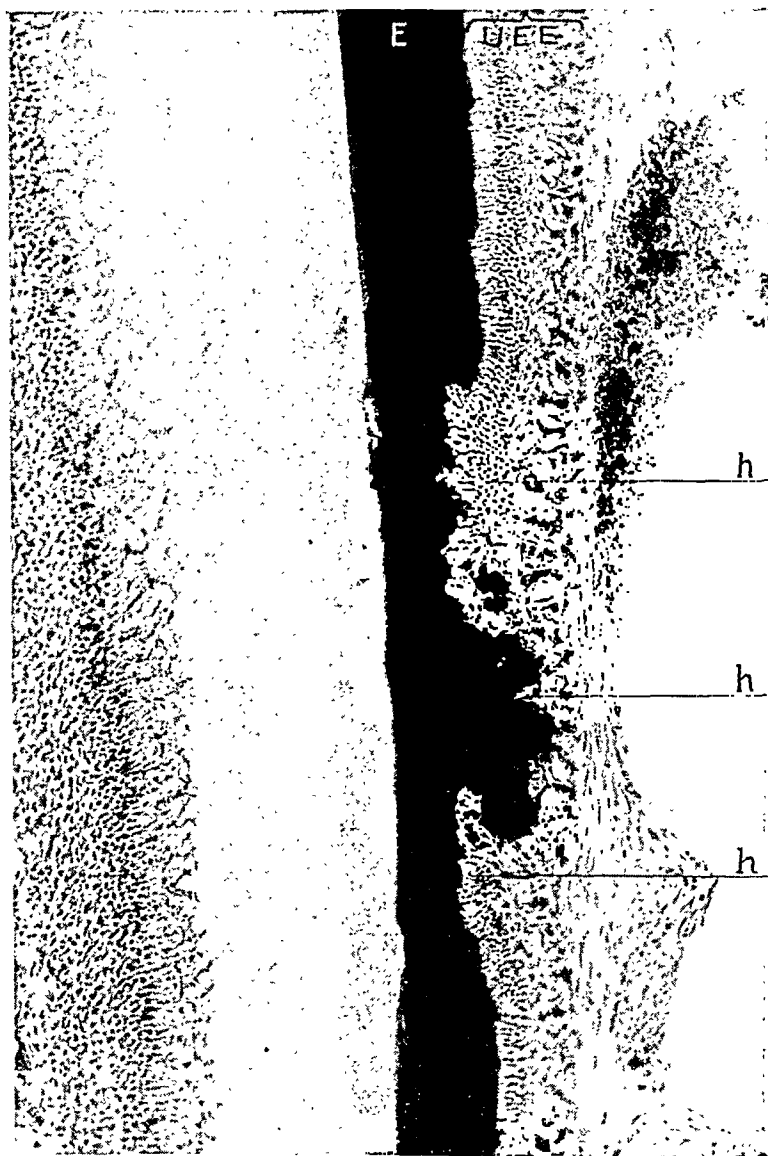


Fig. 10.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-eight days: *h*, area of hypoplasia of enamel; *E*, enamel; *U.E.E.*, united enamel epithelium. The specimen (1262 B) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 150:1.

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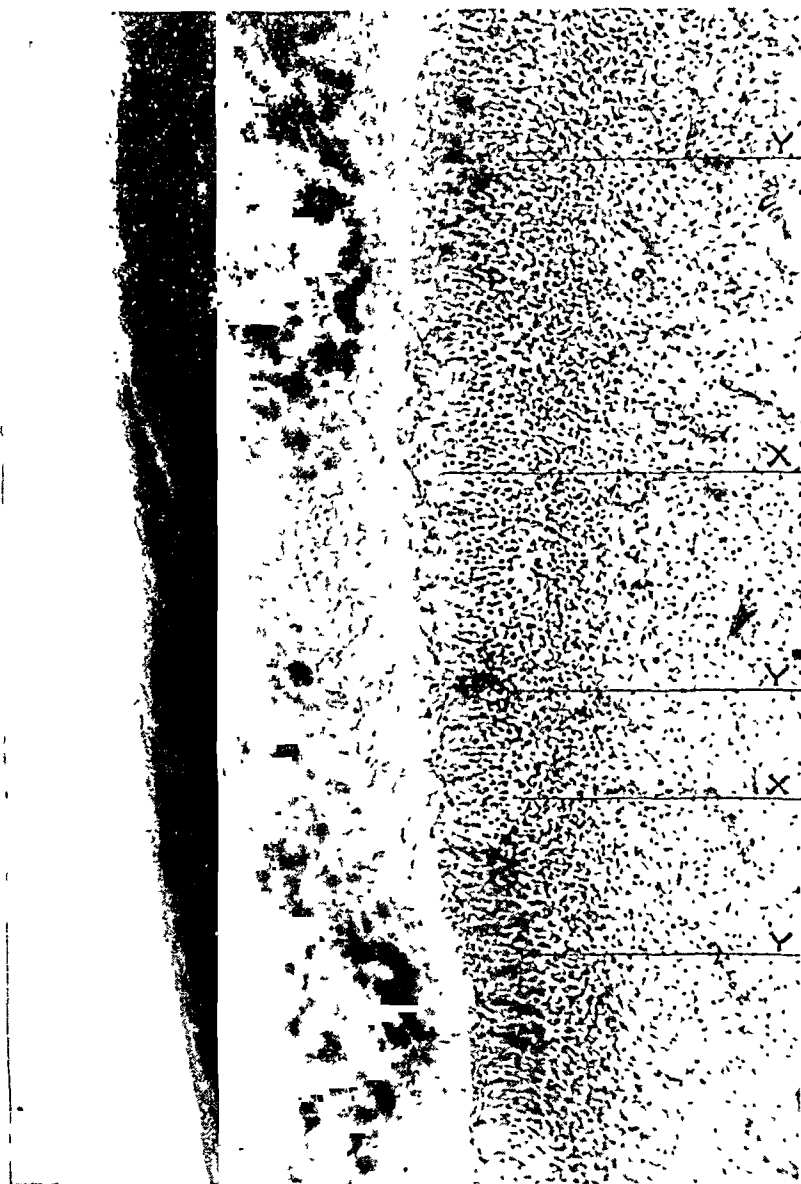


Fig. 9.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-eight days: *x*, group of atrophic odontoblasts; *y*, group of vital odontoblasts. The specimen (1262 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4  $\times$ ; extension of camera, 80 cm.; reduced from a magnification of 150:1.

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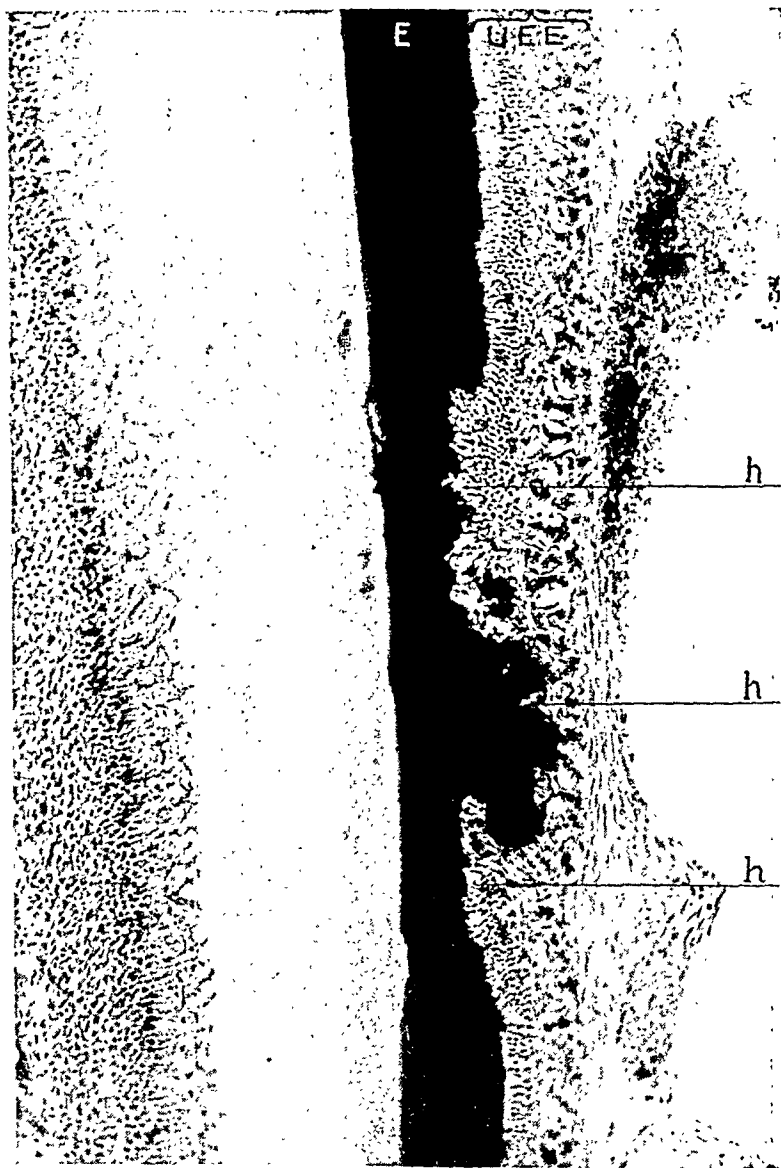


Fig. 10.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for twenty-eight days: *h*, area of hypoplasia of enamel; *E*, enamel; *U.E.E.*, united enamel epithelium. The specimen (1262 B) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4  $\times$ ; extension of camera, 80 cm.; reduced from a magnification of 150:1.

tooth, all from an animal that had been on a rachitic diet for forty-two days. In the upper part of figure 12 conditions are similar to those described in figure 4. However, the zone of predentin is smaller. It seems as if the calcification of the predentin has started again after an interval of only partial or spotty deposition. This is shown by the for-



Fig. 11.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for thirty-five days: *e*, predentin; *x*, group of atrophic odontoblasts; *y*, group of vital odontoblasts; *E*, enamel; *O*, odontoblastic layer. The specimen (1273 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 150:1.

mation of the dense homogeneous layer, *f*, following the zone of irregular calcification, *b*. At the higher magnification this can be seen clearly. The darker staining at *y* shows regeneration and is accompanied by renewal of calcification, between areas of exhausted odontoblasts and spotty deposition. Figures 14 and 15, taken further apically, show that



Fig. 12.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for forty-two days: *a*, normally calcified zone; *b*, zone of irregular globular calcification; *f*, layer of new homogeneous calcification; *y*, vital odontoblasts. The specimen (1282 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective, 1 b; eyepiece, 4  $\times$ ; extension of camera, 80 cm.; reduced from a magnification of 55:1.

the degeneration is more uniform, and that a large number of capillaries are included in the matrix.

It has been mentioned before that the greater portion of the molar teeth of the rats had been formed when these animals were placed on the diet. Some distinct differences are found, however, when figure 16



Fig. 13.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for forty-two days: *a*, normally calcified zone; *b*, zone of irregular calcification; *f*, layer of new homogeneous calcification; *y*, group of vital odontoblasts. The specimen (1282 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 ×; extension of camera, 80 cm.; reduced from a magnification of 150:1.

(taken after seven days) is compared with figure 17 (taken after twenty-seven days). Both pictures have been made at the same magnification, 86:1, as that of the first molars of animals from these groups. Complete growth of tooth and bone had not taken place at the end of the prediet period of twenty-five days. The distinct osteoid, dentinoid and



Fig. 14.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for forty-two days: *b*, zone of irregular calcification; *v*, included blood vessel. The specimen (1282 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4×; extension of camera, 80 cm.; reduced from a magnification of 150:1.

cementoid margins are seen in both cases, but appear more pronounced in figure 17. The root in figure 17 is considerably longer, although the diameter is the same in both specimens. The width of the predentinal



membrane at the crest of the alveolar ridge has been reduced by continued growth of osteoid tissue and the length of the apical predentin, measured from the lowest point of calcified dentin to the apex, is almost twice as great in the older animal.

This might indicate continued rapid growth of dentinal matrix in the apical direction. The same increase of osteoid tissue is also to be noticed at from *r* to *s*, figures 16 and 17. Concerning the development

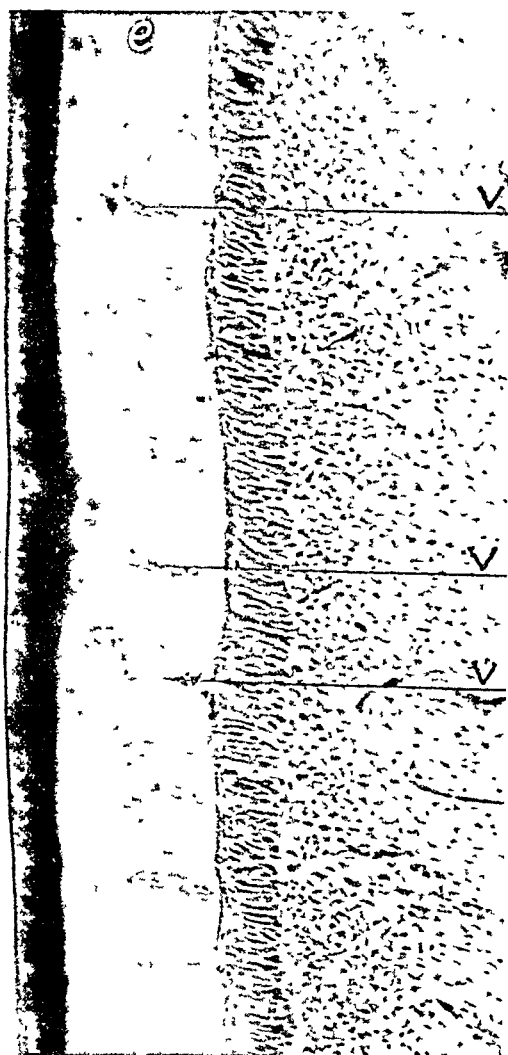


Fig. 15.—Incisor of rat that had been on rachitic diet 3143 (McCollum) for forty-two days: *c*, predentin; *v*, included vessel. The specimen (1282 A) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective; apochromatic lens, 16 mm.; eyepiece, 4 $\times$ ; extension of camera, 80 cm.; reduced from a magnification of 150:1.

and calcification of the dentin in molars, it can be said that after twenty-seven days the calcified area, as well as the predentinal layer, is much wider, with a consequent narrowing of the pulp.

The presentation of more pictures of molar teeth is unnecessary since all specimens from animals on the diet for a period longer than twenty-seven days showed results similar to those that appear in figure 17. This seems to indicate that the molars stop growing in rats at about the fiftieth day of life.



Fig. 16.—Molar of rat that had been on rachitic diet 3143 (McCollum) for seven days: *r*, limit of normal formation of bone; *s*, osteoid tissue. The specimen (1205 B) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective, 1 h; eyepiece, 8 $\times$ ; extension of camera, 62.5 cm.; reduced from a magnification of 86:1.



Fig. 17.—Molar of rat that had been on rachitic diet 3143 (McCollum) for twenty-seven days: *r*, limit of normal formation of bone; *s*, osteoid tissue. The specimen (1249 B) was embedded in a collodion preparation and stained with hematoxylin-eosin; Leitz objective, 1 b; eyepiece, 8  $\times$ ; extension of camera, 62.5 cm.; reduced from a magnification of 86:1.

## COMMENT

That rickets retards the eruption of teeth and interferes with their development is widely recognized. The conception that hypoplasia of enamel occurs only on a rachitic base is no longer tenable (Fleischmann<sup>7</sup>). However, notwithstanding the many publications on the subject, the histogenesis of hypoplasia of enamel is still a subject of controversy.<sup>8</sup>

On account of the similarity in origin and development between bone and dentin, we should expect in experimental rickets to find in dentin changes similar to those in bone. It has been shown that there is a disturbance, or lack of deposition of lime, in dentin, while the matrix is formed normally. Little has been written concerning the rôle of the odontoblasts and the local causes of faulty calcification in rickets.

In order to understand pathologic features in the formation of dentin, it seems pertinent to review the process of its calcification under normal conditions and the rôle of the odontoblasts in this process. In this way we shall assemble better our knowledge and ideas concerning the clinical and histologic picture of the pulp and of the dentin in rickets.

Orban<sup>9</sup> gives a concise summary of what is known regarding the formation of predentin and of the origin and chemical nature of von Korff's fibers. According to him, these originate in the pulp tissue and by a chemical transformation are changed from argyrophil to collagenous fibers.

It seems to be the general conception that the odontoblasts take part in the formation of the matrix by the secretion of the interfibrillar substance (cementing or ground substance). This organic matrix gradually calcifies from the periphery toward the pulp. It is pierced by parallel tubules that extend from the pulp to the outer border of the dentin and contain prolongations of the protoplasm of the odontoblasts (Tomes' processes). Calcification begins around these processes in the form of spherical masses, calcospherites, which gradually fuse, forming a solid mass.

As to the exact mode and channels of secretion of the lime salts in dentin, Mummery<sup>10</sup> stated:

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7. Fleischmann, L.: *Arch. f. mikr. Anat. u. Entwicklungsgesch.* **68**:297, 1906; *Verhandl. d. 5 Internat. zahnärztl. Kong.*, Berlin, 1909.

8. This question will be discussed in a later paper on the basis of this material.

9. Orban, B.: *Dental Histology and Embryology*, ed. 2, Philadelphia, P. Blakiston's Son & Company, 1929; *J. Am. Dent. A.* **9**:1547, 1929.

10. Mummery, J. H.: *The Microscopic and General Anatomy of the Teeth*, ed. 2, Oxford Medical Publications, New York, Oxford University Press, 1924; *Phil. Tr. Roy. Soc. Med.* **182**:527, 1891.

We know that the deposit takes place not in direct contact with the odontoblasts, but gradually advances from above upon the odontogenetic zone. This would suggest that the lime salts pass by dialysis from the tubes of the dentin, containing the protoplasmic prolongation of the secretory odontoblast cell, into the dentinal matrix, and the minute subdivisions of the tubes with their protoplasmic contents would afford a very efficient means of distribution of the calcifying substance within the matrix. It is difficult to explain the deposit of the lime salts at a distance from the odontoblastic cell itself, unless we consider that the dentinal fibril which is an extension of the cell itself takes an active part.

Erdheim,<sup>11</sup> in his classic publication describing the teeth of rats after extirpation of the parathyroids, expressed himself fully about the changes in dentin. From his work it can be seen that the environment of the dentinal tubules is the most active area of the deposition of lime. It is here that deposition begins and it is here that lime remains most highly concentrated. The proximity of 'Tomes' fibrils seems to have an influence on the process of calcification. One gains the impression that the odontoblast takes care of the importation of lime. In this they act in direct contrast to the blood capillaries, which occasionally extend far into the dentin. Around such vessels calcification does not take place. The chemism of the odontoblast, with its prolongation into the tubule, is without doubt, exactly opposite to that of the blood capillaries. If the odontoblast controls the importation of lime, in the last analysis it obtains the salts from the blood; but the direct presence of blood does not permit calcification; rather it hinders this.

The deposition in direct proximity to the dentinal tubules reminds one of the homologous conditions in bone. Pommer<sup>12</sup> described thoroughly a similar action of the deposition of lime in relation to the direct environment of the canals in bone. The conditions near areas of active circulation are unfavorable for the deposition of lime. Only at some distance, where the circulation is slower, are conditions favorable for calcification (Kassowitz<sup>13</sup> and Pommer<sup>12</sup>). This is true of dentin, as well as of bone. Because of the high vascularity of the pulp and of the odontoblastic layer, the zone of dentin nearest the pulp remains free from calcium under normal conditions. Only at some distance from the odontoblastic layer does the dentin calcify. By growth in length, as well as in breadth, the dentinal layers are removed sufficiently from the region of active circulation and thus calcify.

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11. Erdheim (footnote 5, first reference).

12. Pommer, G.: Untersuchungen über Osteomalazie und Rachitis, Leipzig, F. C. W. Vogel, 1885.

13. Kassowitz, M.: Med. Jahrb., 1879, p. 145.

The rôle of the odontoblasts in this process, as Mummery<sup>10</sup> mentioned, has not been definitely determined. In discussing the observations of this author, Tomes<sup>13a</sup> stated:

If the dentin matrix is formed in a connective tissue basis, it becomes a question of what share the odontoblasts take in the process. It may be taken as certain that the odontoblast is concerned in the development of the dentinal tubes . . . by its process, . . . and it seems as if the same odontoblast forms the whole length of the fibril, itself receding backward as the dentin grows. It becomes doubtful if the odontoblasts alone are concerned in elaborating the calcifying material, . . . it (is) probable that they are active agents in the development of the dentin and it is certainly known that the dentinal tubules are not formed in their absence.

In other words little has been proved as to the rôle of the odontoblasts in the formation of predentin or as to their function in calcification.

Orban,<sup>9</sup> in his recent article on the development of dentin, quoted the following statement from Jasswoin,<sup>14</sup> "The odontoblasts already developed do not produce fibers but may play a rôle in the metabolism of the dentin and probably in its calcification."

Orban himself said: "The interfibrillar or cementing substance is most probably the product of the odontoblasts and also other pulp cells."

Von Kórf<sup>15</sup> thus expressed his ideas:

The ivory cells, according to my findings, do not take part in the formation of the ivory ground substance; but they are in such close contact with it that an important function is revealed. This consists in supplying the dentinal layer which is becoming thicker and more densely calcified, with nutritional channels. This is done apparently by developing an increasing number of tooth fibers with ramifying branches. By this rich ramification of fibers, and the dentinal tubules which develop with them, and remain in constant communication with the pulp cavity, an open pipe system is made for the transmission of nutritional fluid.

Summarizing, we may say that, according to present knowledge, the calcification of the ground substance of dentin most probably takes place through the dentinal tubules, or, better, through Tomes' processes (Mummery,<sup>10</sup> Tomes,<sup>6</sup> Orban<sup>9</sup>). The function of the odontoblasts in the formation of dentin matrix consists in forming dentinal tubules and probably in producing the interfibrillar or cementing substance. A definite proof of these points thus far has not been given. It seems pertinent at this point also to review briefly the ideas of various authors concerning the modus of pathologic changes in the dentin.

Fleischmann<sup>7</sup> was one of the first to study rachitic changes in dentin. Starting with the normal calcification of dentin, in which the formation of the matrix and its calcification are parallel, so that the width

13a. Tomes, C. S.: *A Manual of Dental Anatomy*, ed. 7, Philadelphia, P. Blakiston's Son & Company, 1914, p. 195.

14. Jasswoin, G.: *Arch. f. mikr. Anat. u. Entwicklungsmechn.* **102**:291, 1924.

15. von Kórf, K.: *Ergebn. d. Anat. u. Entwicklungsgesch.* **17**:247, 1907; *Arch. f. mikr. Anat.* **67**:1, 1905; *Anat. Anz.* **64**:383, 1928.

of the uncalcified zone (predentin) is nearly always constant, he described his histologic observations in rachitic teeth. Here the calcification no longer runs parallel with the formation of the matrix. This results in an enlargement of the uncalcified zone, which is wider where growth is most active. Where growth has been completed, as at the tip of the incisor tooth, the calcification continues, and one finds a predental layer of normal width. Of course, one finds a return to normal conditions when the rachitic process has been overcome.

In rickets the globules of lime are essentially smaller and are not deposited as densely. As a result, large numbers of small interglobular spaces occur. It is emphasized by Fleischmann, that in rickets one is dealing with a disturbance of the calcification of dentin parallel with the normal function of the dentinal ground substance and without any influence on the odontoblastic layer. He confirmed these observations also in the teeth of rats after parathyroidectomy.

The observation that the odontoblastic layer remains intact during the formation of dentin has been confirmed by Siegmund and Weber.<sup>16</sup> However, they described severe cases, which showed an involvement reaching the stage of complete degeneration of the odontoblastic layer.

Toyofuku<sup>17</sup> reported that after parathyroidectomy in rats the odontoblasts undergo severe changes parallel with the dentin. The cells become atrophied, and their epithelial-like arrangement is greatly disturbed. In those areas where capillaries and even pulp tissue grow into the dentin, the line of the odontoblasts is completely interrupted, or at least the cells cannot be recognized as odontoblasts.

Euler and Meyer<sup>18</sup> said:

In dentin the formation of dentinoid tissue is continued for a time after calcification has ceased. When the dentinoid is no longer calcified the differentiation of the odontoblasts is lost and with this the formation of new matrix.

This leads us to ask the question: Does the degeneration or atrophy of the odontoblastic layer (Siegmund and Weber,<sup>16</sup> Toyofuku<sup>17</sup>) occur as the result of the systemic disorder (avitaminosis), or does the differentiation of the odontoblasts, and with it the new formation of the ground substance, stop because the calcification of the matrix ceases?

In discussing the changes induced in the dentinal layer of our own animals, we must differentiate between the several pathologic stages, all of which increase in severity during the course of the experiment.

Primarily there is a distinct increase in the width of the predental layer seen after seven days (fig. 1), which indicates that calcification no

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16. Siegmund, H., and Weber, R.: *Pathologische Histologie der Mundhöhle*. Leipzig, S. Hirzel, 1926.

17. Toyofuku, T.: *Frankfurt. Ztschr. f. path. Anat.* 7:2, 1911.

18. Euler, H., and Meyer, W.: *Pathohistologie der Zähne*, Munich, J. F. Bergmann, 1927.

longer parallels the formation of the matrix. The primary zone of calcification is distinctly marked off from the irregular zone formed during the experiment. This is clearly shown in all sections. The width of the globular, insufficiently calcified layer is almost the same in all specimens. This proves that in all animals the precipitation of lime salts stopped after a short period on the deficient diet. The changes in the odontoblastic layer are of great importance in explaining these observations.

As early as fourteen days (fig. 2) atrophic changes occur in the odontoblasts. These changes become more pronounced as the experiment continues.

After twenty-one days (fig. 3) larger areas of the odontoblastic layer have degenerated, and paralleling these calcification has stopped. This can be observed even more clearly in figures 7, 8 and 9, taken after twenty-eight days. It is important to note in these pictures that the structure of the predental layer is normal.

However, a different picture is seen in some other animals, although they have been on the diet for the same length of time. As figures 4, 5 and 6 show, marked pathologic changes in the structure of the dentinal matrix are found accompanying the atrophy of the odontoblasts. Here some vessels are included in the matrix, which seems to indicate that occasionally certain odontoblasts have not functioned properly. These degenerative changes are seen to be especially pronounced in figure 6; the odontoblastic layer can hardly be recognized. This is accompanied by marked changes in the matrix and the inclusion of innumerable blood vessels, a process that can be explained by the failure of the odontoblasts to form cementing substance and Tomes' fibers; a space is thus created in which the capillaries are left, being enclosed by the continued formation and cementing of the matrix on either side under the influence of active cells. These inclusions are possible only because the odontoblasts discontinue their activity in these places. Figures 4, 5 and 6 show the odontoblasts to be uniformly involved, and cells or groups of cells with definite vitality, as seen in figures 7, 8 and 9, are no longer seen.

In comparing these six pictures it is apparent that animals on the diet for approximately the same length of time can show marked differences in structure and in degenerative changes. These observations indicate that the involvement of the odontoblasts shown in figures 4, 5 and 6 must have been more severe and must have taken a much more rapid course than that shown in figure 7, for instance, in which groups of cells still show vitality. These variations probably depend on constitutional differences in the individual animals.

Whereas the inclusion of blood vessels was found occasionally in animals that had been on the diet for twenty-eight days, this condition was met with regularly in the group that had been on the diet for forty-



two days. In other words, if the destructive action of the deficient diet is allowed to act over a sufficient length of time, forty-two days or longer, and the animal does not die, the odontoblastic layer undergoes a progressive degeneration beginning at many points. This produces the typical picture of vital and nonvital cells (figs. 7, 8 and 9), until complete degeneration has taken place as shown in figures 14 and 15.

If the blood vessels do not all degenerate simultaneously, they become enclosed. If the diet leads to an early irregular, but complete, elimination of certain odontoblasts, owing to lack of resistance, capillaries may be found included as early as twenty-seven or twenty-eight days.

Summarizing, we may state that in the experiment with diet 3143, the odontoblasts suffer severely in vitality, so that their activity gradually decreases until they are completely atrophied.

It has been stated that when the activity of the odontoblasts ceases, the calcification of the corresponding matrix also stops. This proves that the calcification of the dentinal matrix depends on the activity of the odontoblasts.

In comparing figures 4 and 5, we may conclude from the width of the predentinal layer and the zone of faulty calcification, that in the incisal portion (fig. 4) the odontoblasts remained vital for a longer period of time. This seems to answer the question asked in the first portion of this paper: Is the degeneration of the odontoblasts the expression of lack of available lime salts and of consequent atrophy because of inactivity, or are the odontoblasts primarily damaged and so incapable of functioning even in the presence of sufficient lime salts?

The photomicrographs show clearly that in experimental rickets one is dealing with a primary involvement of the odontoblasts. This involvement is not the result of a lack of available lime salts, but is due rather to a deficiency of vitamin D. Furthermore one can see that in the incisal portion calcification was still going on after it had ceased nearer the apex. This shows that lime salts were available in the blood. If one considers that the circulation in the apical region is greater, the failure of calcification in this area can be explained only on the basis of a lack of some substance in the cells that enables them to utilize the lime salts. The incisal odontoblasts are older and were formed earlier than those nearer the apex. The older cells probably have a larger reserve of vitamin D, which enables them to remain active after the younger cells have ceased to function. Also some cells in the apical areas, as in figures 7 and 8, are active in the deposition of lime salts, which further proves that these salts are present in available form. If groups of cells degenerate, lack of calcium is not responsible, but rather the exhaustion of cellular vitamin with no replacement from the diet. The cells atrophy, and calcification ceases.

According to our observations, this primary degeneration takes place in the odontoblasts of all animals that have been on this diet fourteen days or longer. Because of the similarity in origin and function of the odontoblasts and the osteoblasts, it would seem that the changes in formation of bone (osteoid margins and other structural irregularities) are parallel to the observed changes in dentin. In other words, the pathologic changes of the bones in rickets are due to a primary degeneration of the osteoblasts. We believe this involvement to be due to the lack of vitamin D. This directly affects the vitality of these cells and seems to prevent them from utilizing the lime salts, thus stopping calcification.

The degeneration can occur uniformly throughout the pulp; however, it generally begins near the apical portion and proceeds toward the incisal portion (figs. 4 and 5). In most cases it occurs in groups which seem to develop progressively. The mountain-like points of calcification shown in figures 7, 8 and 9 illustrate this. Finally it ends in a complete and uniform degeneration of the whole layer. If single cells are exhausted early and others continue active, the described inclusion of blood vessels occurs.

Furthermore, from these observations the conclusion may be drawn that the odontoblasts are the chief factors in the development of the dentin. They form Tomes' fibrils, which serve to transport the calcifying material, and by the deposition of cementing substance in connection with von Korff's fibers, they form the homogeneous matrix, bordered by a layer of very fine capillaries. Any disturbance in the odontoblastic layer during development leads to alteration in the formation of the matrix, as well as in its calcification.

Our material shows conclusively that the cementing substance is produced by the odontoblasts.

As stated before, the degeneration of the odontoblasts in the incisal area occurs later than nearer the apex. In figures 12 and 13 it can be seen that the incisal cells regenerate more rapidly. Zone *b* represents new, homogeneous calcification superimposed on an area of irregular deposition. This is found first in the incisal portion. Inclosed blood vessels in the incisal show that severe, if not complete, degeneration of the odontoblasts occurred. The recurrence of calcification indicates that the atrophic odontoblasts have renewed their activity.

Extensive experiments in this direction would possibly produce interesting results which might be of great value in treating patients with dental conditions arising from rickets.

#### CONCLUSIONS

A rachitic diet (McCollum's, 3143) produces marked pathologic changes in the dentin if the diet is maintained during the development of the teeth.

These changes increase in extent and severity as the period of the diet is prolonged.

The changes induced are analogous to those produced in the long bones under the same dietary conditions.

In the early stages the changes consist of a diminution in the amount of calcium salts deposited, even after such a short period as seven days; a change in the type of calcification, from a homogeneous to an irregular, globular type; the continued formation of dentinoid matrix, which appears to widen as a result of the slowing down of the calcification.

Atrophy of the odontoblasts appears after about fourteen days, increasing in severity as the feeding experiment progresses. This is found first in the most recently formed cells, spreading slowly to the older odontoblasts. Since the diet is deficient only in vitamin D, it is believed that this primary atrophy of odontoblasts develops when all the cellular vitamin D is utilized.

Because of the similarity in function of odontoblasts and osteoblasts, it would seem that the changes in bones in rickets are probably due to a primary involvement of the osteoblasts.

Certain marked changes result from the odontoblastic atrophy; complete arrest of calcification in contrast with continued deposition of lime salts by adjacent vital odontoblasts; in cases in which single odontoblasts degenerate, no cementing substance is formed, and capillaries become included by the continued formation of matrix by the adjacent vital cells.

On the basis of the data presented it is believed that the odontoblasts are chiefly responsible for the formation of dentin. They are required to produce the cementing substance which holds von Korff's fibers in place. Their presence is necessary to form Tomes' processes and so give nutrition and calcifying material to the dentin, and furthermore in order that calcium salts may be deposited and the matrix calcified.

# ISOLATED GIANT GROWTH OF A BRANCH OF THE PULMONARY ARTERY ASSOCIATED WITH CONGENITAL BRONCHIECTASIS

REPORT OF A CASE \*

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At autopsy in a case of squamous cell carcinoma of the penis, an excessive overgrowth of a branch of the left pulmonary artery was found. No report of a similar case could be detected in the literature on anomalies of the blood vessels or lungs.

Its significance lies in its substantiation of Huntington's<sup>1</sup> theory that the postbronchial arterial plexus is the precursor of the pulmonary artery.

## REPORT OF CASE

*History.*—A white man, 69 years of age, had noticed a rapidly growing tumor on the penis for six months. Biopsy revealed a squamous cell carcinoma. Because of the absence of metastases, the penis was removed surgically. A periurethral abscess developed, with generalized septicemia, and the patient died from sepsis and bronchopneumonia ten days after the operation.

In the past history no reference was made to pulmonary symptoms, and physical examination of the chest revealed nothing abnormal, except terminally, when bronchopneumonia was manifested.

*Autopsy.*—On postmortem examination (by R. H. Jaffe) metastases were not found, but the organs showed severe parenchymatous degeneration as a result of the periurethral abscess.

The heart, aorta and lungs will be described in detail because of their relation to the giant artery.

The heart weighed 310 Gm. (the patient weighed 124 pounds [55.2 Kg.]). The wall of the left ventricle measured 12 mm. and that of the right ventricle 3 mm. The myocardium was a dirty reddish brown and friable.

The aorta 1 cm. above the cusps measured 90 mm. and presented small calcified plaques above the valve and in the thoracic portion, especially about the openings of the intercostal arteries. In the pleural cavities there were focal fibrous adhesions about the apexes of both lungs.

The right lung was distended and heavy. The upper and middle lobes were crepitant, while the lower lobe was subcrepitant. The surfaces made by cutting were deep purplish red and moist with blood and frothy fluid.

The upper lobe of the left lung was crepitant; the edges were boggy, and the surfaces made by cutting were dark gray. In the apex was a deeply anthracotic area, 20 mm. by 15 mm., with a light gray caseated center, 2 mm. in diameter.

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\* From the Department of Pathology, Cook County Hospital; Dr. Richard H. Jaffe, director.

1. Huntington, G. S.: *Am. J. Anat.* **27**:99, 1920; *Anat. Rec.* **17**:165, 1919.

The lower lobe of the left lung was subcrepitant, except for the posterior and lowermost portion, which was noncrepitant. The cut surfaces of the lower lobe were deep purplish red. The consolidated area, on sectioning, was dark red, and at the upper border of this area was a cavity, 15 by 10 by 10 mm. in diameter, which was filled by a thick, honey-like, light-gray mucus and was lined by a light-gray, shining membrane. The branch of the pulmonary artery leading to this area was rigid and measured 8 mm. in diameter. Because of this peculiarity, the lung was cut into many sections perpendicular to the giant artery, so that its relations could be studied carefully.

It was found that this artery originated in the middle of the lower lobe from a branch of the pulmonary artery and immediately took a sharp turn of 90 degrees to course posteriorly, inferiorly and medially. It gradually approached the posterior surface of the lung and at the diaphragmatic aspect was covered only by the visceral pleura of the diaphragmatic and posterior surfaces of the lung.

The vessel became gradually larger after its bend and 1 cm. below this angle measured 8 mm. in diameter; from this point it again became smaller and at its subpleural termination was 3 mm. in diameter. The entire length of the artery was 6 cm.

The wall of the vessel was 1.5 mm. thick, and the intima was studded with atherosclerotic plaques. The lumen was occluded by a reddish-gray thrombus.

The bronchiectatic cavity followed a course similar to that of the artery. Cranially, it lay ventral and lateral to the artery. Caudally, it wound about the artery anteriorly and assumed an anterior and medial position.

*Microscopic Examination.*—The Artery: The wall of the artery in the cranial portion (3 mm. in diameter) was slightly hypertrophic and was composed of three coats, an intima, a media and an adventitia. The artery lay adjacent to the medial and anterior aspect of the bronchus. Extending caudally, the vessel gave off several branches that corresponded to the bronchi. These vessels showed a structure similar to that of the parent artery. Gradually the vessel became larger and passed behind the dilated bronchus, forming an angle of 90 degrees, and assumed a position lateral and dorsal to the bronchiectatic channel. Before its bend the vessel presented subendothelially a focal area of necrosis, with pus cells, which extended into the media. A branch of this vessel showed similar changes.

At its widest portion (8 mm. in diameter), the vessel was composed of all three coats characteristic of an artery. The media was markedly thickened and contained an abundance of elastic fibrils; the internal and external elastic membranes were fairly distinct. The internal elastic membrane was thickened and split. The muscle fibers were hypertrophic. The intima showed advanced atherosclerotic changes, as evidenced by large crescentic areas that occupied from one third to one half of the circumference of the vessel and were composed of fatty acid crystals, debris and calcific deposits. At one point, continuity of the intima was interrupted, and a thrombus had formed at this site, which filled the entire lumen of the vessel. The thrombus was composed of fibrin, leukocytes, mononuclear cells and red blood corpuscles. The media in the region of this sclerotic plaque was thin and edematous. Branches leading from this portion of the vessel showed similar changes, many being obliterated by thrombi.

The terminal portion of the vessel extended to beneath the pleura and constituted about one half of the cross-sectioned area of the lung at this point. Its most distal portion ended as a blind pouch with collapsed walls. The latter was almost entirely replaced by fibrous tissue and beneath the pleura was lost in dense connective tissue bands.

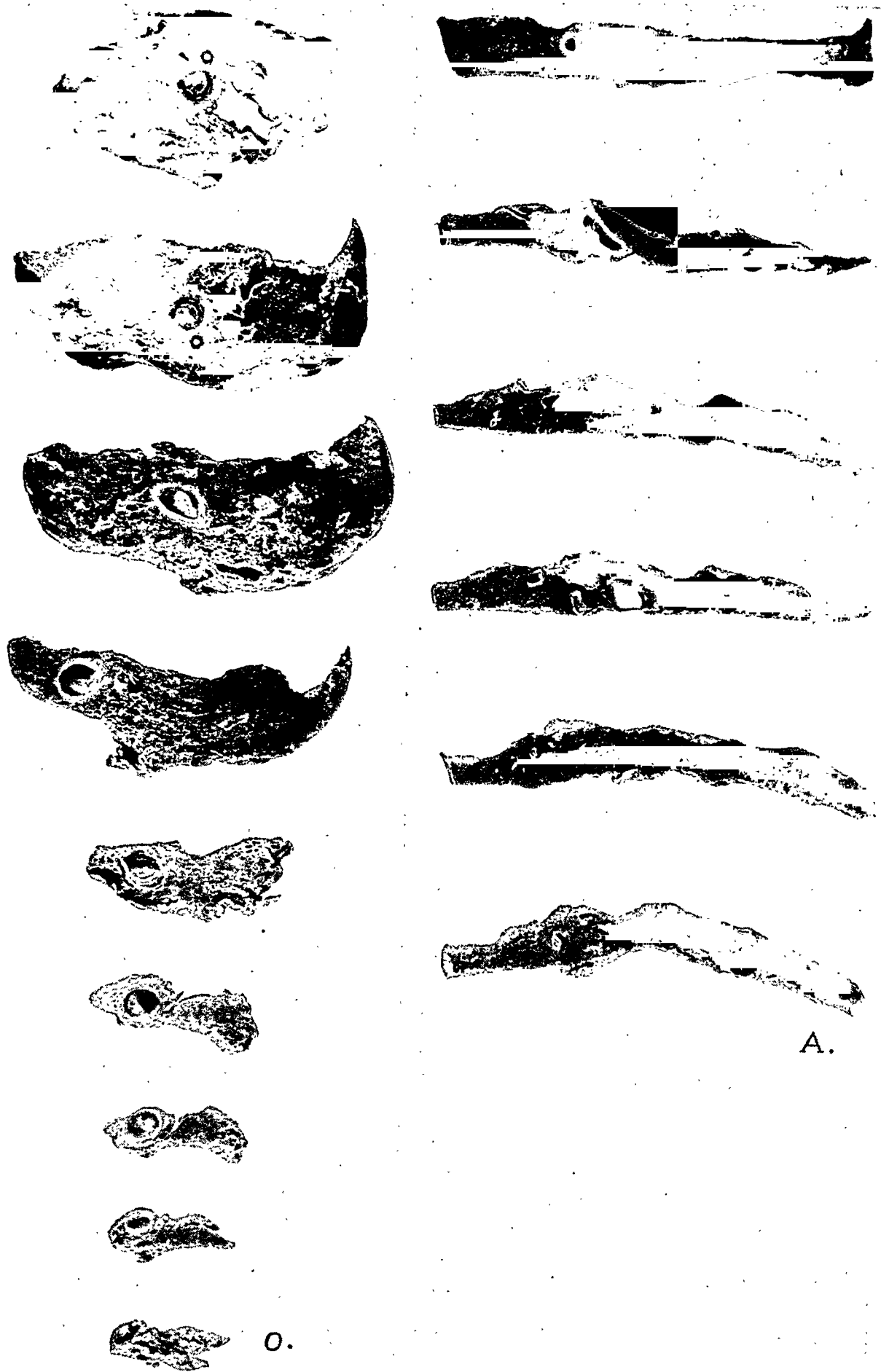


Fig. 1.—Cross-sections of the giant artery and the bronchiectasis: *A.*, portion nearest the hilus of the lung; *O.*, blind subpleural termination of the artery. The sections are arranged in order; about three-fourths natural size.

**Veins:** About the cranial portion of the artery, the veins were found on the posterior and lateral aspect of the bronchi, being histologically unchanged. With the appearance of the giant artery, no corresponding vein could be found. There were numerous smaller veins that accompanied the smaller bronchi and arteries. These, too, showed no abnormal changes.

**Bronchi and Bronchioles:** In the region of the cranial portion of the giant artery, the bronchi and bronchioles were markedly distended, and their walls were thin. The epithelial lining was cuboidal. The mucosae were intact. The walls were composed mainly of connective tissue and a few muscle and elastic fibers. The lumina were filled with a homogeneous substance staining pale blue (hemalum and eosin).

There was a slight amount of fibrous tissue surrounding the bronchi, but cellular infiltration was not present.

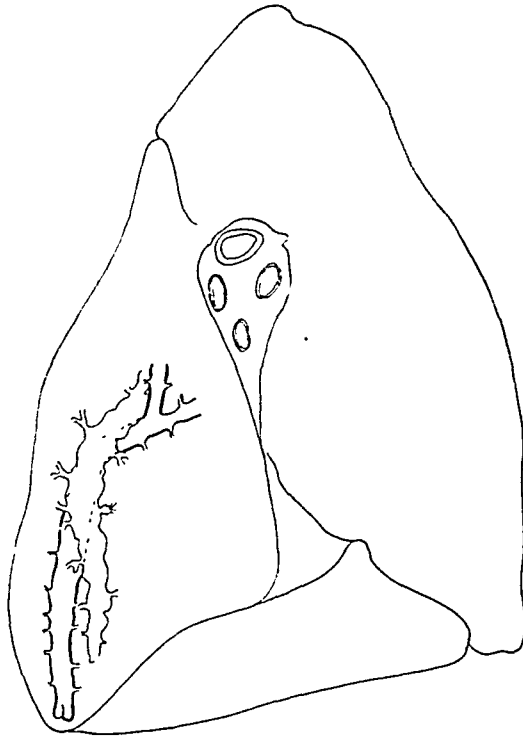


Fig. 2.—Schematic drawing showing relationship of the artery and the bronchiectasis. The heavy line outlines the artery; the fine line, the bronchus.

Caudally, several bronchiectatic cavities, each of which measured 10 mm. in diameter, seemed to have fused. Only one artery and vein accompanied these cavities; hence the latter represented numerous sacculations of one bronchus.

This dilated bronchus extended anteriorly and medially, above the artery in the form of a continuous dilated tube with fusiform swellings. The wall had become somewhat thickened as a result of an increase in fibrous tissue. The epithelium lining the mucosa became high cuboidal, and under oil immersion cilia were seen. A few glands were scattered beneath the mucosa. No evidence of cartilage was noted.

The bronchus gave issue to several branches along its course, which showed a similar histologic structure.

The alveoli about the cranial aspect of the artery were thin-walled and dilated, and many were filled with blood. In the region of the giant artery and the

bronchiectatic cavity the alveoli were somewhat compressed. As the terminal portion of the artery was approached, there was a gradual diminution in the number of alveoli; their walls here were collapsed and thickened. At the termination of the blood vessel, the alveoli disappeared and were replaced by dense fibrous

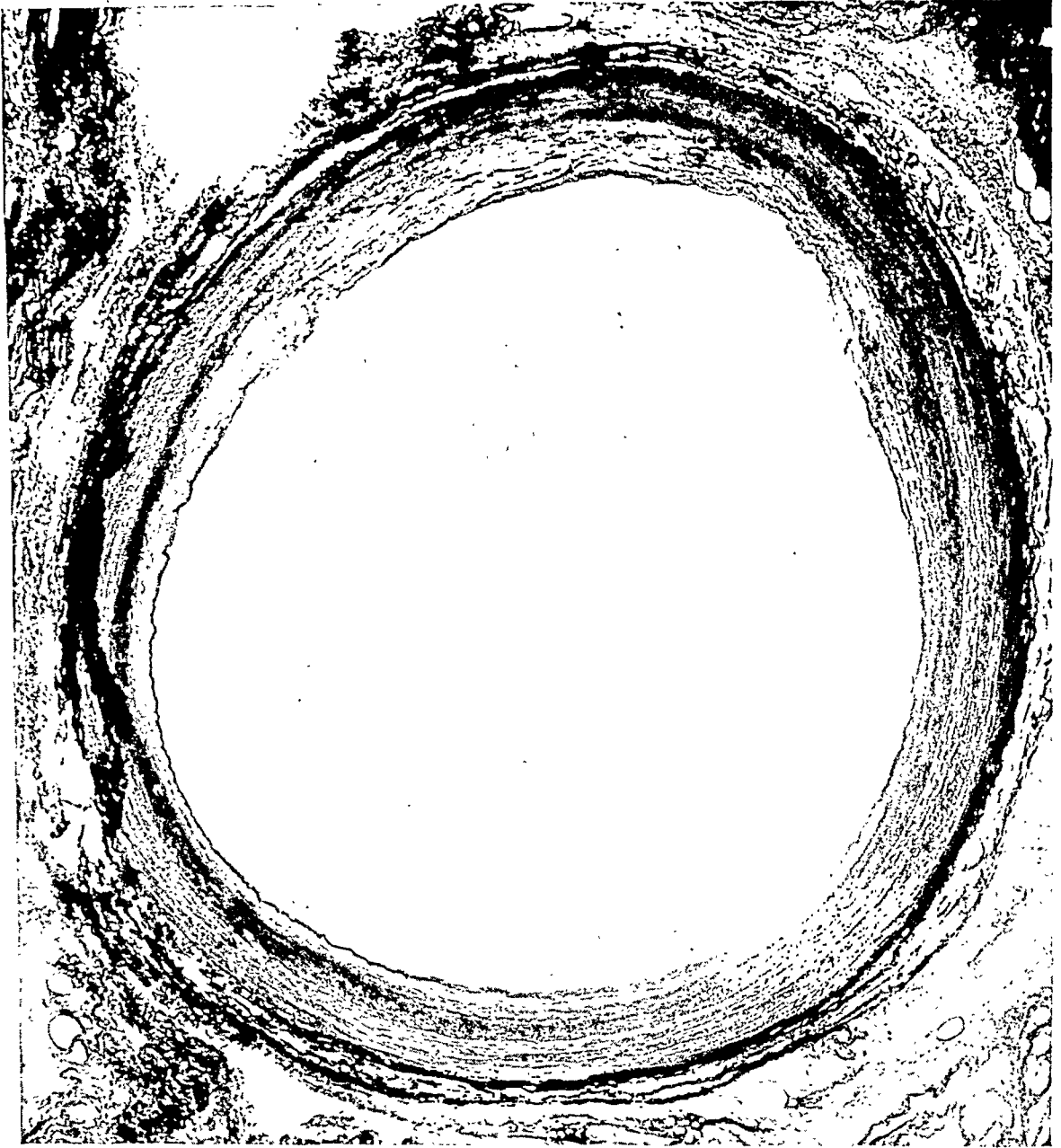


Fig. 3.—Wall of the giant artery showing its three coats. Note the internal elastic membrane and the thick media rich in elastic fibers; elastica stain;  $\times 25$ .

tissue with large vacuoles resembling fat cells. Numerous widely dilated capillaries were scattered throughout this portion of the lung. Coal pigment, which was abundant in other parts of the lung, was practically absent in this area.





## SUMMARY OF CASE

From a histologic standpoint the giant vessel described appeared to resemble a mature hypertrophic artery. The atherosclerotic changes, however, were marked, much more so than in the aorta or in the medium-sized blood vessels.

The giant artery originated from a pulmonary artery, 3 mm. in diameter, in the substance of the lung and terminated blindly in the subpleural region. The relation of this vessel to a corresponding bronchus was dorsal and lateral, a condition normally present in the lung (Miller<sup>2</sup>).

The bronchus corresponding to the giant vessel was the seat of a bronchiectasis which began with the origin of the artery, but which terminated 1 cm. proximal to the termination of the vessel. The branches issuing from the bronchus were similarly dilated. The wall of the bronchus was thin and contained no cartilage. The lining epithelium was high, cuboidal and ciliated. Several mucous glands were found in the submucosa.

The alveoli in the region of the origin of the artery were of normal histologic appearance, but became collapsed as the termination of the vessel was approached, finally being completely replaced by fibrous and fatty tissue. The absence of anthracosis and of inflammation in this portion of the lung was notable.

## PATHOGENESIS

As to the origin of the giant artery, many possibilities present themselves.

Hypertrophic arteries may be found in chronic inflammatory tissue, but the absence of inflammation and the huge dimensions of the vessel speak against this mode of origin.

Vicarious hypertrophy and hyperplasia of an artery may develop if collateral channels are taxed as a result of an increased demand for function of a part. No such demands were made in this case; there was a decrease rather than an increase in function.

The possibility of this vessel's being a bronchial one, originating from the aorta or from one of the intercostal arteries which became severed from its source, is also improbable, as this vessel accompanied a bronchus, after the manner of a pulmonary vessel, and finally communicated, by a branch 3 mm. long, with the pulmonary artery. The

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2. Miller, W. S.: *Arch. f. Anat. Entwicklungs-gesch.*, 1900, p. 157; *Am. Rev. Tuberc.* 2:659, 1918-1919.

latter factor would not disprove its being a bronchial artery, as held by Nicolas,<sup>3</sup> although Miller<sup>2</sup> denied the existence of anastomosis.

The exclusion of the aforementioned factors leaves only one possibility, that of a congenital anomaly.

As a result of the work of His<sup>4</sup> and Zimmermann,<sup>5</sup> the pulmonary arteries have been described as originating from the ventral portion of the sixth arterial arch of each side and descending as a complete and continuous vessel to supply the lung. If this vessel were to undergo a congenital hyperplasia, it would be expected to include the entire vessel, or the proximal portion more likely than the distal one, contrary to the condition that was present in the reported case.

More recently (1919), however, Huntington,<sup>1</sup> in studying the development of the pulmonary artery in the cat (embryos measuring from 4 mm. to 6 mm. from crown to rump) found that the methods previously described were erroneous. His conclusions were that the pulmonary artery develops independently of the sixth arch by the differentiation of a distinct arterial channel in the ventral portion of the postbronchial pulmonary plexus. The latter is derived from a series of irregular channels (seventh to twelfth dorsal aortic branches) which open into the dorsal aorta. The sixth arch serves merely as the point of junction at which, after coalescence with the pulmonary plexus, the blood is carried from the ventral segment of the sixth arch to this prepared channel of the pulmonary artery.

With this conception as to the origin of the pulmonary artery, the case reported can more readily be explained, in that a congenital malformation of a segment of this postbronchial pulmonary plexus might lead to a giant artery of a distal branch of the pulmonary artery.

Conydon,<sup>6</sup> however, in studying human embryos of the same size, failed to find a double primordium from the pulmonary artery, although Ingalls,<sup>7</sup> in a human embryo, measuring 4.9 mm., succeeded in doing so.

Indeed, from ontogenic considerations, the work of Huntington<sup>1</sup> is substantiated. Thus Simpson<sup>8</sup> described two cases in human beings in which lobes of the lung were supplied by branches of the aorta, and Paul<sup>9</sup> described two similar cases. In one of these cases, he

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3. Nicolas, A.: Poirier's *Traité d'anatomie humaine*, Paris, L. Battaille & Cie, 1895, vol. 4, p. 528.

4. His, W.: *Arch. f. Anat. u. physiol. Anat.* **2**:89, 1887.

5. Zimmermann, W.: *Anat. Anz.* **4**:720, 1889.

6. Conydon, quoted by Keibel and Mall: *Human Embryology*, Philadelphia, J. B. Lippincott & Company, 1912, vol. 11, p. 482.

7. Ingalls, N. W.: *Arch. f. mikr. Anat.* **70**:506, 1907.

8. Simpson, G. C. E.: *J. Anat. & Physiol.* **42**:221, 1908.

9. Paul, Fritz: *Virchows Arch. f. path. Anat.* **267**:295, 1928.

injected a thin barium sulphate paste into the vessels and was able to demonstrate a direct communication between the anomalous vessel and the pulmonary artery.

These authors did not offer sufficient explanations for the presence of these anomalous arteries, but it is most likely that they are persistent branches of the postbronchial plexus, which had failed to separate from the dorsal aorta.

In the reported case, a similar condition existed, except that a severance from the dorsal aorta occurred, perhaps at a late stage, after full development of the artery.

The close proximity between the giant artery and the bronchus is evidence that the former was in some way responsible for the bronchiectasis. The mechanism of the formation of the bronchiectasis may be explained in one of three ways:

First, the large vessel may have compressed the proximal portion of the bronchus, with the result that there was a retention of secretion with a dilatation of the bronchus. At no place could such a relationship be found, although a honey-like, grayish-white secretion was present in the bronchus.

Second, the constant pressure of the large vessel may have stimulated growth of the larger bronchi in the form of a true tumor (fetal bronchial adenoma of Störk<sup>10</sup> and also Hondo<sup>11</sup>) or as a focal or universal bronchiectasis (Grawitz,<sup>12</sup> Meyer<sup>13</sup>). In such instances the cartilage proliferates, and the alveoli remain fetal or do not develop at all. These possibilities were excluded in that the alveoli were present and fully matured. They decreased in number and finally disappeared in that portion of the lung where the area of the vessel was greater than that of the remaining lung parenchyma. Then, the histologic picture of the bronchus was that of hypoplasia rather than that of proliferation. The walls were thin, with a marked decrease in the amount of elastic and muscle fibers; the epithelium lining the mucosa was composed of a single layer of ciliated cuboidal cells, and cartilage was absent. The third and most plausible possibility is that with the establishment of the pulmonary circulation the pressure exerted by the giant artery caused a collapse of the surrounding alveoli. With the inspiration of air, the bronchus leading to this part was inflated, but the cushion-like uniform counteraction of the alveoli was absent, so that a dilatation of the bronchus occurred. This process, continuing over many years,

10. Störk, O.: *Wien. klin. Wchnschr.* **10**:25, 1897.

11. Hondo, T.: *Centralbl. f. allg. Path. u. path. Anat.* **15**:129, 1904.

12. Grawitz, Paul: *Virchows Arch. f. path. Anat.* **82**:217, 1880.

13. Meyer, H.: *Virchows Arch. f. path. Anat.* **16**:78, 1859.

led to a stretching and rupture of the elastic and muscle fibers and their replacement by fibrous tissue.

The collapse of the alveoli was followed by a thickening of their walls and replacement by fibrous tissue. That this collapse occurred at birth is borne out by the absence of coal pigment.

Inflammation as a possible factor for the bronchiectasis is excluded by the presence of an intact mucosa with ciliated epithelium and the absence of pleural adhesions about this portion of the lung.

The explanation of the marked atherosclerotic changes in the giant artery may be based on an exaggeration of the sclerotic process present in the other large and medium-sized arteries. That this vessel suffered more than the others is perhaps due to its blind termination, which increased the peripheral resistance and augmented the physiologic degenerative processes (Rosenthal<sup>14</sup>).

The thrombus in the vessel was recent and formed after the generalization of the infection. The factors responsible for the thrombus were: a colloidal, chemical or physical alteration of the blood as a result of the sepsis (Dietrich<sup>15</sup>); a sluggish circulation because of the marked parenchymatous degeneration of the myocardium; a localization in this artery as a result of the severe atherosclerotic changes and rupture of the intima (Rosenthal<sup>14</sup>).

#### SUMMARY

There is described a giant artery of the left lower pulmonary lobe that communicated with the pulmonary artery and terminated blindly in the subpleural region. The histologic picture was that of a hypertrophic artery with advanced sclerotic changes.

The giant vessel was determined to have originated in a persistent branch of the postbronchial arterial plexus. Its congenital origin was emphasized by its retainment of pronounced arterial characteristics in adult life.

An accompanying bronchiectasis resulted from the pressure exerted by the artery on the surrounding alveoli, which compressed them and caused ectasia of the bronchus due to lack of opposition.

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14. Rosenthal, S. R.: Sclerosis of the Pulmonary Artery and Arterioles: A Clinical Pathological Entity, *Arch. Path.* **10**:717, 1930; Thrombosis and Embolism, *J. Lab. & Clin. Med.* **16**:107, 1930.

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# GENERALIZED ACTINOMYCOSIS (PYEMIC FORM)

REPORT OF A CASE \*

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The lesions of actinomycosis have been described as occurring in almost every organ of the body. The process spreads from any one of a number of possible primary foci of infection, usually by direct continuity. It restricts itself to no anatomic boundaries, but erodes anything within its path. If not extending by direct continuity, however, the organisms seem to travel almost entirely by way of the blood stream (Nathan<sup>1</sup>).

Rarely are lymphatic channels used. Lymph nodes are generally avoided. Only the perineural lymphatics are sometimes involved in metastases to the brain and spinal cord (Werthemann<sup>2</sup>). The lesions are themselves very vascular. They have a special affinity for blood vessels, and break freely into all those that they meet. The organisms are difficult to cultivate from the blood stream during life, but Shrewsbury<sup>3</sup> once did so successfully. The following case of pyemic actinomycosis demonstrated the predilection of the process for hematogenous spread.

## REPORT OF A CASE

*Clinical History.*—A colored man, a laborer, 45 years old, with no special occupational exposure, was admitted to the hospital with a chronic, progressive cough of seven weeks' duration. It was productive of much yellowish-white, foul sputum streaked with blood. He had frequent night sweats. He complained also of a pain in the right side of the chest and of a constant "misery" just under the right shoulder blade. There was marked weakness in the left hand and numbness in the right.

His temperature was hectic, with daily variations from 99 to 104 F. He had many carious teeth. Over the right upper pulmonary lobe, there were impaired resonance, increased tactile fremitus and diminished breath sounds. The left lung was clear. The x-ray picture showed a dense infiltration in the upper third of the right pulmonary field. Just to the right of the spinal column, close to the inferior angle of the right scapula, there was a bulging, tender, nonpulsatile, slightly fluctuant mass. On aspiration, a thick purulent, foul material was obtained. Repeated

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\* Submitted for publication, March 3, 1931.

\* From the Department of Pathology of the Cook County Hospital.

1. Nathan, H.: *Klin. Wchnschr.* **33**:1543, 1930.

2. Werthemann, A.: *Virchows Arch. f. path. Anat.* **255**:7 and 9, 1925.

3. Shrewsbury, F.: *M. Rev.* **3**:372, 1928.

cultivations of it gave no growth. Smears showed degenerated polymorphonuclear leukocytes and degenerated cocci, but no definite organisms.

The clinical diagnosis was empyema necessitatis with pulmonary tuberculosis.

Examination of the sputum for tubercle bacilli repeatedly gave negative results. Three days after incision of the abscess mass, the patient suddenly suffered two attacks of clonic spasm involving the left arm and the left side of the head. A diagnosis of secondary abscess of the brain was considered. In the course of the next four weeks, numerous metastatic abscesses appeared in the subcutaneous tissues of the left forearm, the left hip, the right leg, the chest, the back and the abdominal wall. Then for the first time, and only because of the unabating persistence of the pyemic spread, was the possibility of actinomycosis considered.

*Necropsy* (by Dr. R. H. Jaffe).—In addition to the subcutaneous abscesses described, there were small abscesses in and about the thyroid gland, in the left side of the dome of the diaphragm, in the liver, in the left kidney and in the anterior wall of the left cardiac ventricle. Pus-filled polypoid masses projected from between the papillary muscles of the mitral cusps. Between the upper pole of the right kidney and the inferior aspect of the liver, there was a cavity, 9 cm. in diameter, filled with thick, light yellow pus. The upper pole of the kidney was entirely replaced by discrete and confluent cortical abscesses, which extended into the medulla. The right renal vein was occluded by a light yellow-gray, adherent blood clot, which was centrally liquefied.

The pleural cavity on the right side was obliterated by dense, fibrous adhesions. Near the apex there was a plum-sized, pus-filled cavity which communicated by a descending sinus with the right interscapular abscess described. The upper part of the right pulmonary lobe was firm, and surfaces made by section were dark gray, with fine, yellowish-white lines radiating about small, dilated bronchi, which contained thick, yellow pus. The lymph nodes at the bifurcation of the trachea were but slightly enlarged.

In the galea aponeurotica there were two abscesses that covered irregular defects in the skull and were filled with pus. The sphenoid bone also contained irregular, pus-filled cavities. In the sagittal sinus, a purplish-gray, partly liquefied thrombus was adherent to the wall.

The subdural and subarachnoidal spaces over the right cerebral hemisphere were filled by thick, light yellowish-green pus. In the right parietal lobe, involving the medulla and part of the cortex, there was an irregular cavity, 3.5 by 4 by 2.5 cm., filled by thick, yellow pus.

*Microscopic Examination*.—In the areas of carnification in the upper lobe of the right lung, there were numerous small abscesses that seemed to originate from the small bronchi. The abscesses were lined by a vascular granulation and filled by polymorphonuclear leukocytes and larger, fat-filled mononuclear cells. Embedded with these cells were numerous clusters of radiating mycelia with club-shaped thickenings of their peripheral ends.

The cerebral abscesses contained many pus cells and large mononuclear cells. In the pus, there were single, distinct, ray-shaped clusters and more loosely arranged, branched, slender mycelia. About the clusters there was a cloudlike, light blue radiating zone (fig. 1). The adjacent tissue was loosened and cellular.

In the kidney, the large, wedge-shaped area of diffuse suppuration contained many typical clusters. The arciform and interlobar veins corresponding to this area were filled by pus and numerous star-shaped bodies (fig. 1).

*Bacterioscopic Examination*.—Star-shaped clusters of long, slender and branched, gram-positive threads, some with a club-shaped thickening of the free

end, were found in smears from the diaphragmatic abscess, the brain, the heart and the thyroid gland.

On Herrold medium under anaerobic conditions a growth of gram-positive, slender, branched mycelia was obtained from the diaphragmatic abscess. Pure culture failed because of an overgrowth of *Proteus vulgaris* and cocci.

*Anatomic Diagnosis.*—The anatomic diagnosis included: chronic indurative-suppurative actinomycosis of the right upper pulmonary lobe; generalized actinomycosis (pyemic form) with abscesses in the thyroid gland, myocardium,

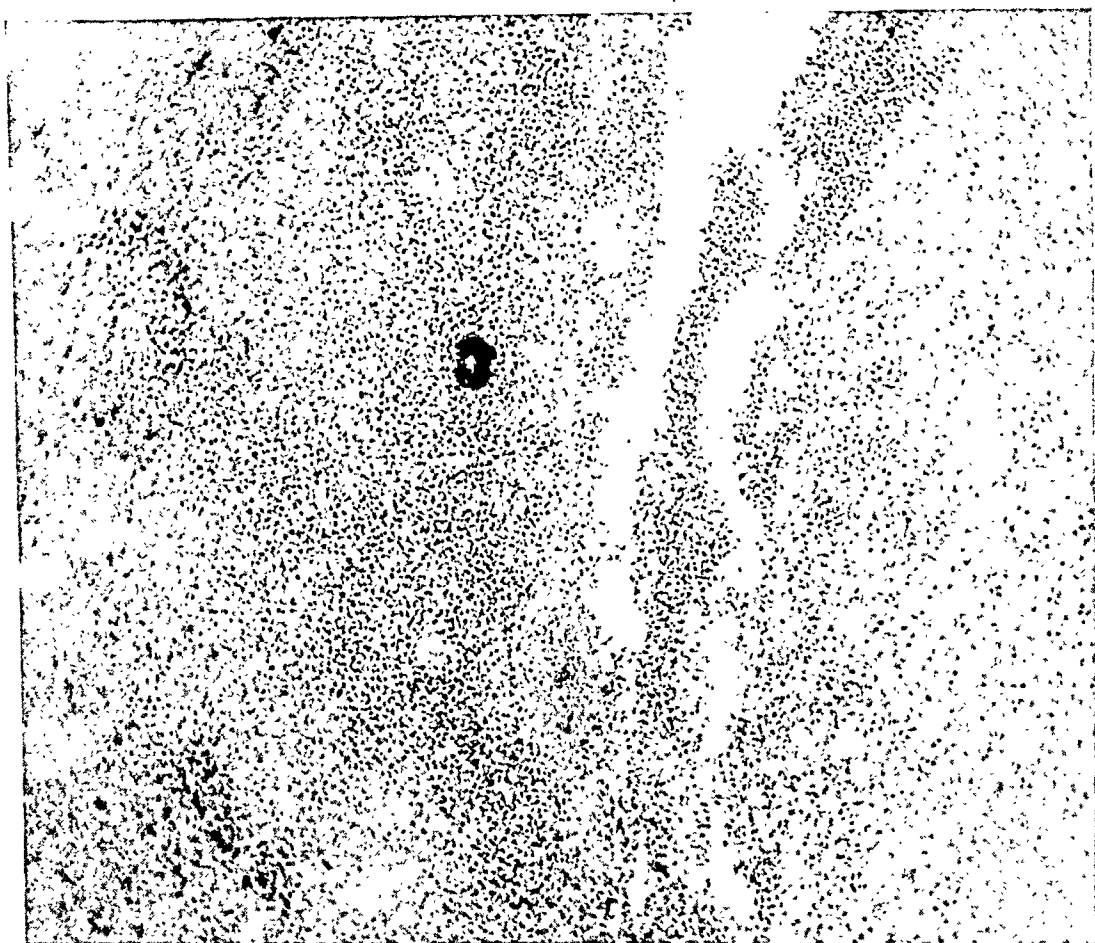


Fig. 1.—Renal actinomycotic thrombophlebitis. The interlobar vein in the right kidney is involved in thrombophlebitis with two colonies of actinomycetes in the center. Elastica stain was used to show the remaining outlines of the vein, and the outline of the accompanying renal artery. Renal tissue is seen above and below. Leitz objective; apochromatic lens, 16 mm.; periplanar, 6.

diaphragm, kidneys, liver, galea aponeurotica, brain and subcutaneous tissues; actinomycotic thrombophlebitis of the right renal vein and sagittal sinus; suppurative internal pachymeningitis and suppurative leptomeningitis of the right cerebral hemisphere; suppurative osteomyelitis of the occipital, parietal and sphenoid bones, and perinephritic abscess of the right side.



## COMMENT

Actinomycosis is caused by a branching, filamentous fungus of the order *Microspinales* and family *Nocardiaceae* (Castellani and Chalmers,<sup>4</sup> Castellani,<sup>5</sup> Wolff and Israel<sup>6</sup>). The genus that is most commonly associated with the disease in man is *Cohnistreptothrix israeli*. It is anaerobic and difficult to cultivate. It is not found in nature, but has been found in carious teeth of even normal persons, whence it may invade its host (Lord,<sup>7</sup> Naeslund,<sup>8</sup> Turner,<sup>9</sup> Warwick<sup>10</sup>). It forms characteristic colonies with a central mycelial mass and radiating peripheral filaments arranged like the petals of a daisy.

A few cases are caused by *Actinomyces bovis* of Bostroem.<sup>11</sup> This is an aerobic organism, common in nature, but only rarely found in carious teeth (see Wright<sup>12</sup>). The infection here is directly exogenous. Identical clinical syndromes may occasionally be produced by other organisms, such as *Actinobacillus lignieresii* and even staphylococci (Magnusson<sup>13</sup>). None of these organisms, however, produce the typical radiating colonies of *Cohnistreptothrix israeli*.

Dresel<sup>14</sup> divided the nocardioses into two groups. He restricted the term "actinomycosis" to infections with the *cohnistreptothrix*. He applied the term "streptothricosis" to all cases caused by the aerobic, noncolonizing members of *Nocardiaceae*, by the actinobacillus or by other organisms. According to this classification, the case here reported, because of the presence of the characteristic colonies and the anaerobic growth, is a true actinomycosis.

The primary focus of infection is usually in carious teeth (60 per cent), but it may be in the lungs (14 per cent) or in the intestines (18 per cent) (see Sanford and Magath,<sup>15</sup> Zininger<sup>16</sup>). Actino-

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5. Castellani, A.: *Fungi and Fungous Diseases*, Chicago, Am. Med. Assn., 1926.

6. Wolff, M., and Israel, J.: *Virchows Arch. f. path. Anat.* **126**:11, 1891.

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12. Wright, J. H.: *J. M. Research* **13**:349, 1905.

13. Magnusson, H.: *Acta path. et microbiol. Scandinav.* **5**:170, 1928.

14. Dresel, E. G.: *Beitr. z. path. Anat. u. z. allg. Path.* **60**:185, 1915.

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mycosis of the lung may be primary, bronchial, arising by inspiration or aspiration of infected material through the airway. It may be secondary, arising either by direct extension from the head and neck or from the abdomen, or by way of the blood stream from some distant focus.

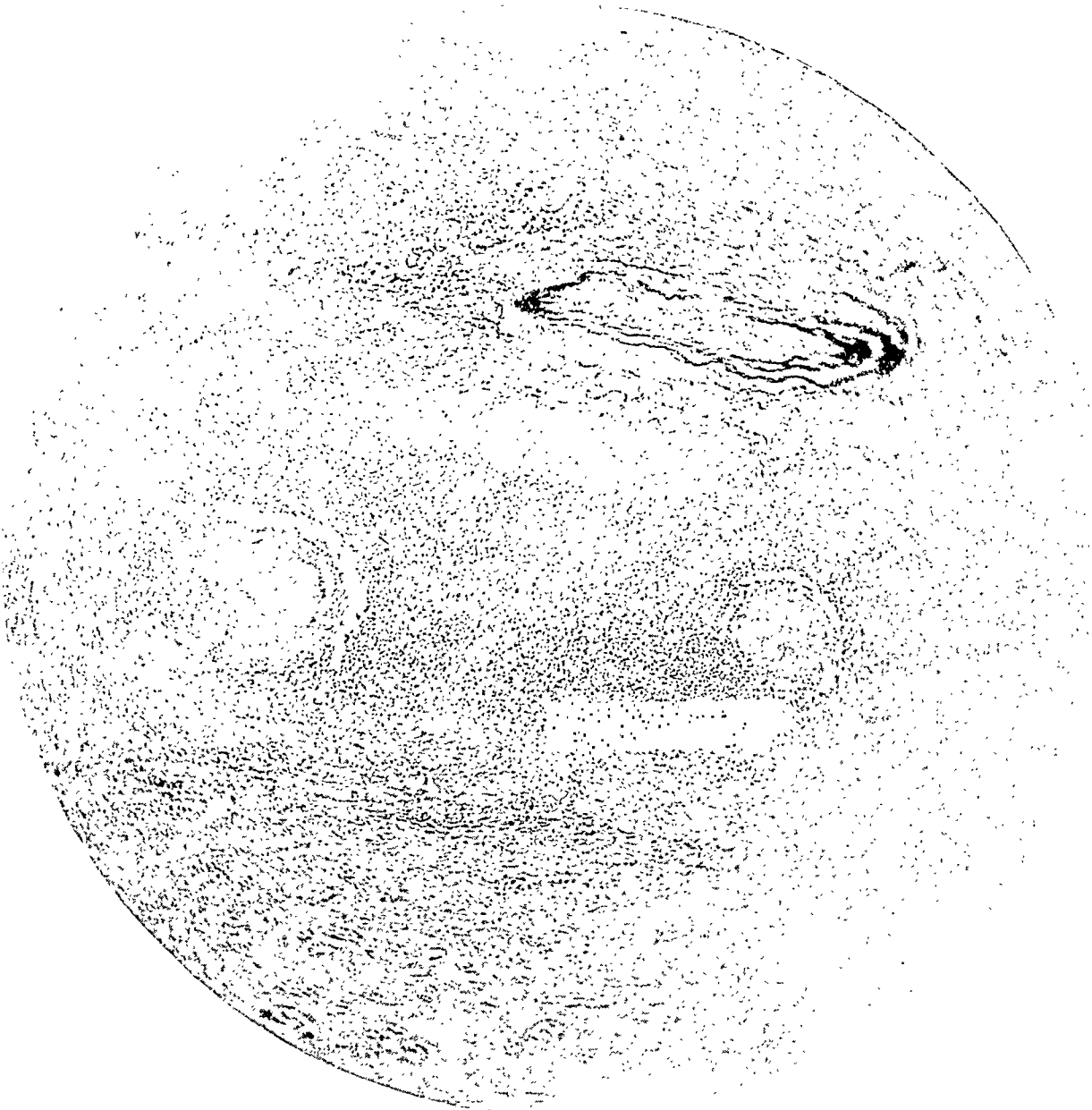


Fig. 2.—An actinomycotic abscess of the brain is shown, with brain tissue to the right and an abscess with a single colony of actinomycetes on the left. Under higher power many single mycelial threads could also be seen. Leitz objective; apochromatic lens, 16 mm.; periplanar, 6.

Primary pulmonary infection starts as a simple, actinomycotic, catarrhal bronchitis. It progresses by peribronchial infiltration to form

chronic bronchopneumonic patches, which are characterized by whatever is the varying balance between necrosis with formation of cavity and the marked tendency to dense perifocal fibrosis (Marko,<sup>17</sup> Koch<sup>18</sup>). Most commonly the process starts in the base of the lung. In this case it started in the apex.

Pleural extension leads to a serous or richly vascular granulomatous reaction, with the formation of encapsulated empyemic cavities (see Chambers,<sup>19</sup> Lord,<sup>20</sup> Geymueller<sup>21</sup>). When situated paravertebrally, these abscesses compromise the adjacent cervical and intercostal nerves and thus give rise, even without involvement of vertebrae or the cord, to ipsilateral neurologic symptoms and signs. In this case, the pleural abscess at the apex of the right lung and the perithyroid abscesses had produced pain in the right side of the chest and numbness in the right arm. The localized empyema finally pointed to the outside and broke through. It may, when more closely situated to them, break through the diaphragm or into the mediastinum, or even into the heart (Doyle,<sup>22</sup> Preston<sup>23</sup>).

The typical sulphur granules are readily overlooked in sputum or in pus, if certain precautions are not taken. The material must be washed by vigorous shaking in sterile distilled water or broth. The granules sink to the bottom of the tube, whence they are picked out, crushed between slides and stained by the Gram and the Ziehl-Neelsen methods. The material should also be grown on dextrose-agar or broth, aerobically and anaerobically, to differentiate *Cohnistreptothrix* from the other organisms belonging to *Nocardiaceae*. Secondary infection in the abscesses rapidly diminishes the chances of demonstrating the mycelia. Biopsy of the granulation tissue in the wall of the abscesses would then prove more successful.

Wherever it sets up a footing, actinomycosis produces very vascular lesions and also induces local thrombophlebitis. The lesions erode and break into any available local blood vessel. Dissemination occurs to a single organ or to many organs. Wherever they land, the organisms set up similar lesions and again attack the local blood vessels, to multiply the chances for spread.

Hematogenous spread usually starts from the lungs, but it may start from the mouth or from the gastro-intestinal tract (Jacoby<sup>24</sup>).

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22. Doyle, C. D.: Lancet **2**:600, 1927.

23. Preston, T. W.: Brit. M. J. **2**:1172, 1928.

24. Jacoby, F.: Arch. f. klin. Chir. **149**:621, 1928.

In a case described by Nathan,<sup>1</sup> primary actinomycosis of the appendix led to actinomycotic pylephlebitis with hepatic abscesses that broke into the hepatic veins to set up secondary abscesses of the lung. These, in turn, led to pulmonary actinothrombophlebitis, but death occurred before further dissemination could take place. Hematogenous generalization, starting in the lungs, displays no great preference for any particular organ. The brain, liver, kidneys and spleen are most commonly involved; the myocardium, voluntary muscles, bones, dura, skin and gastro-intestinal tract are less frequently affected, and the thyroid gland, pancreas, thymus and lymph nodes, most rarely. In the florid, pyemic cases no rules of selection are followed.

Actinomycotic foci in the liver may arise by direct extension from adjacent lesions, or by hematogenous spread from the gastro-intestinal tract by way of the portal vein or from the lung by way of the hepatic vein. Numerous cases of all three types have been described (Shoemaker,<sup>25</sup> Greenwell,<sup>26</sup> Lehmann and Kahlstorf,<sup>27</sup> Fulton and Scheppe<sup>28</sup>). In the case here reported, there was no direct extension from adjacent foci, and there were no lesions in the gastro-intestinal tract. The hepatic abscess in the portobiliary septum arose by spread from the lungs by way of the hepatic artery.

The renal foci, too, were hematogenous, and from the lungs. The urinary tract is a relatively frequent site for actinomycosis (Polayes and Lederer,<sup>29</sup> Cummings and Nelson,<sup>30</sup> Kleinschmidt<sup>31</sup>). Clinically, it simulates tuberculosis or tumor of the kidney. The diagnosis is rarely made correctly before operation, but has been established by demonstrating the ray fungus granules in the urine (Beregoff<sup>32</sup>), in pus from the suppurating areas, in currettings from the sinuses or in the involved tissues. The secondary focus in the right kidney in this case had broken into the perinephric space, and had also induced a secondary renal actinothrombophlebitis from which the organisms could be fed back into the lungs. There were as yet, however, no secondary abscesses of the lung.

Actinomycosis of the bones in man is unquestionably rare. In man, "lumpy jaw" is not osteomyelitic, but is an infection of soft

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25. Shoemaker, F.: U. S. Vet. Bur. M. Bull. **2**:391, 1926.

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tissue and a periodontitis from carious teeth (Kaufmann<sup>33</sup>). A few cases of actinomycosis occurring in bones have been reported, arising by direct implantation or by local extension, but most commonly by hematogenous spread (Sartory, Meyer and Meyer;<sup>34</sup> Krogius;<sup>35</sup> Lemon;<sup>36</sup> Simpson and McIntosh.<sup>37</sup>). In this case the bones of the skull had been reached by the hematogenous route, for there were no other local, extracranial lesions to serve as a local source.

Cerebral lesions occur in only 2 per cent of all cases of actinomycosis. Moersch<sup>38</sup> collected forty-eight cases of actinomycosis with involvement of the brain, and a few cases occurring in the spinal cord (Snoke<sup>39</sup>) have also been reported. Jacoby<sup>24</sup> distinguished four types of cerebral actinomycosis: meningitis, encapsulated abscess, cerebral tumor of gelatinous granulations (rare) and combination types. The usual type is that of chronic meningitis, but abscesses are not infrequent.

The brain is usually reached by direct extension from some actinomycotic focus in the head or neck. It is also accessible by extension along the perineural lymphatics. In the absence of any primary local lesion, as in this case, it is reached only by way of the blood stream. The abscess had produced the paresis of the left arm and the final clonic spasms.

From all these abscesses, as well as from the myocardial foci, the blood stream was finally flooded with mycelia, and crop after crop of actinomycotic abscesses appeared in the subcutaneous tissues all over the body.

#### SUMMARY AND CONCLUSIONS

A case of generalized, florid, pyemic actinomycosis is reported, which demonstrates the predilection of actinomycetes for hematogenous spread. The lymphatic system is spared. There is a marked tendency to the development of local actinomycotic thrombophlebitis, and when generalization does occur, it does so practically only by way of the blood stream.

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# BARTONELLA ANEMIA IN NONSPLENECTOMIZED RATS \*

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AND

BURTON L. ZOHMAN, M.D.

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It is a well established fact that within from four to ten days after the removal of the spleen of a rat, anemia develops with a heavy infection of the red cells by *Bartonella*. The consensus is that the rat has, prior to operation, a latent bartonella infection and can control it. As soon as the spleen is removed, the defensive mechanisms of the body are disturbed, and the rat becomes a victim of bartonella infection. It is thought that with the removal of the spleen, a large part of the reticulo-endothelial system is eliminated and that the remaining part is not sufficient to prevent the development of the disease. Various investigators have therefore undertaken experiments to ascertain whether it is possible to produce the disease, not by the removal of the spleen, but by blockade of the reticulo-endothelial system.

Haendel and Haagen<sup>1</sup> found that in eight of twenty-four rats blocked with india ink, a more or less strong bartonella infection developed. Friedberg<sup>2</sup> found that after repeated intravenous injections of 5 per cent india ink, a bartonella infection of the erythrocytes developed without the symptoms of anemia. Cannon and McClelland<sup>3</sup> found that extensive blockade of rats with india ink produces anemia without the appearance of bartonellas in the red cells. The results of these experiments are not sufficiently clearcut in view of the fact that some investigators were able to get a slight bartonella infection of the red cells without anemia, and the others, an anemia without bartonella infection. In an effort to obtain more effective results, we combined blockade of the reticulo-endothelial system of nonsplenectomized rats, with the additional injection of bartonella-infected material. For our experiments, we used a strain of adult rats that had a latent bartonella infection.

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\* From the Department of Laboratories, United Israel-Zion Hospital.

\* This work was done under the direction of Dr. Max Goldzieher.

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## TECHNIC

*Method of Blockade.*—An intraperitoneal injection of 2 cc. of 10 per cent solution of Higgin's india ink in sterile physiologic solution of sodium chloride was given three or four times at two day intervals.

*Preparation of Infectious Material.*—Rats were splenectomized, and within from four to ten days, a heavy bartonella infection with anemia developed. At the height of infection, under aseptic conditions, blood of the rat's heart was obtained in a tube containing sodium oxalate, and an emulsion of the cardiac muscle and liver in saline solution was made by grinding in a mortar.

## RESULTS OF BLOCKADE OF THE RETICULO-ENDOTHELIAL SYSTEM AND INJECTION OF INFECTIOUS MATERIAL INTO RATS

Seventeen nonsplenectomized adult rats were blockaded with india ink injected intraperitoneally and subsequently were infected with infectious material in the same manner. In thirteen anemia developed with bartonella infection of the red cells, and in four bartonella infection developed without anemia. In repeated control experiments, normal, nonblockaded rats of the same strain did not react to the injection of infectious material. The most extensive bartonella infection and anemia were obtained when a total quantity of from 6 to 8 cc. of a 10 per cent solution of the ink was given in a period of from three to six days. The disease developed in most instances in four days, and at the latest in eight days, after injection of the infectious material. The following protocol illustrates a typical case:

Feb. 5: Rat given 2 cc. of 10 per cent solution of india ink intraperitoneally.

Feb. 7: Rat given 2 cc. of 10 per cent solution of india ink intraperitoneally.

Feb. 10: Rat given 2 cc. of 10 per cent solution of india ink intraperitoneally.

Feb. 12: Rat given 3 cc. of blood and emulsion of splenectomized rat having severe bartonella infection and typical anemia.

Feb. 14.—Moderate anisocytosis and poikilocytosis and an occasional bartonella were seen.

Feb. 15: Moderate anisocytosis and poikilocytosis, with a few bartonellas were seen.

Feb. 17: Moderate anisocytosis and poikilocytosis, some polychromasia and heavy bartonella infection were observed.

Feb. 18: Marked poikilocytosis, moderate polychromasia, many normoblasts and very marked bartonella infection were observed. Hemoglobin was 22 per cent; red blood cells, 1,200,000; white blood cells, 33,100; polymorphonuclears, 80 per cent; small lymphocytes, 12 per cent; large lymphocytes, 8 per cent.

*Relapses Following Blockade.*—Three splenectomized rats that had recovered from the typical anemia and severe bartonella infection were given, twelve days after the disappearance of the parasites, injections of 2 cc. of 10 per cent india ink intraperitoneally, from two to five times at two day intervals. In each case, from two to fourteen days after the last injection, a relapse of the disease occurred with severe bartonella infection and intensification of the anemic blood picture.

## EFFECT OF TRYPANOSOMES ON BARTONELLA INFECTION

Mayer<sup>4</sup> noted the occasional appearance of the rods of *Bartonella muris* in the cells of mice and rats infected with trypanosomes. Marmorston-Gottesman and Perla<sup>5</sup> observed that infections of normal adult rats by *Trypanosoma lewisi* are accompanied by a moderate anemia during the first week of infection with the appearance in small number of the bartonella bodies in the red cells. In order to study the influence of trypanosome infection on the development of bartonella infection, we performed three series of experiments using a strain of *Trypanosoma lewisi* which was found accidentally in one of our experimental rats and which was maintained by transfer from rat to rat.

In the first series, seventeen normal adult rats received intraperitoneal injections of blood containing trypanosomes and bartonellas taken at the height of the disease from trypanosome-infected, splenectomized rats. Two animals died in four days; twelve showed, in addition to a trypanosome infection, a bartonella infection of the red cells, and of these twelve, seven presented also the typical anemia; three were not affected.

In the second series, three trypanosome-infested rats were given intraperitoneal injections of india ink. After the blockade, the number of trypanosomes increased tremendously and, in addition, a bartonella infection of the red cells appeared. The animals died in from five to seven days.

In the third series, six splenectomized rats were given, two weeks after they had recovered from the bartonella infection and anemia, an intraperitoneal injection of 2 cc. of oxalated blood containing a great number of trypanosomes. Within from twenty-four to forty-eight hours, a relapse with a very heavy bartonella infection was observed.

HISTOLOGIC CHANGES IN BLOCKADED, BARTONELLA-INFECTED RATS  
(DR. M. GOLDZIEHER)

The changes observed in the tissues of the organs of the rats that were blockaded and then infected were practically identical with those described by Schwarz<sup>6</sup> in his comprehensive survey of the lesions in splenectomized animals. The most characteristic lesion was focal necrosis of the liver (fig. 1). The foci of necrosis were irregularly scattered; their size varied as much as their location. Often endothelial cells were still well maintained in the necrotic areas. Occasionally, leucocytic infiltration was met with in the peripheries of the necrotic foci. Besides these lesions, changes of the Kupffer cells were regularly met

4. Mayer, M.: Arch. f. Schiffs- u. Tropen- Hyg. **25**:150, 1921.

5. Marmorston-Gottesman, J., and Perla, D.: J. Exper. Med. **52**:129, 1930.

6. Schwarz, L.: Folia haemat. **39**:133, 1929.



with. Most of these cells, of course, contained a varying amount of india ink, and many of them appeared considerably increased in size (fig. 2). Those Kupffer cells, however, that contained little or no pigment frequently showed swelling of both cytoplasm and nucleus and occasional phagocytosis of red cells. Many of the Kupffer cells contained iron, which was also demonstrable in the liver cells, particularly in those in the central areas of the lobules.

Necroses similar to those of the liver were demonstrable in the bone marrow (fig. 3). Some of these were apparently of more recent origin

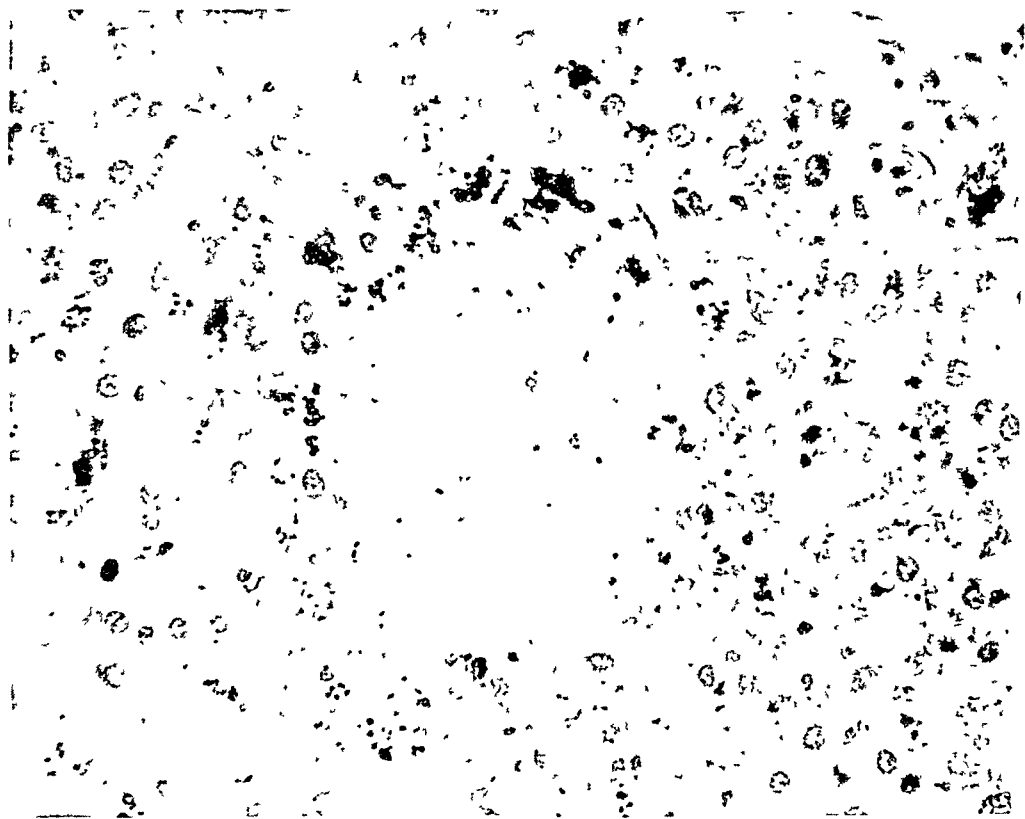


Fig. 1.—Focal necrosis of the liver. Note the accumulation of india ink in the periphery of the necrotic area.

and still showed the outlines of the decaying cells intermingled with amorphous débris and some fibrin.

The lesions of the spleen were not uniform in all the cases; in some of them, excessive congestion and even hemorrhagic infiltration of the pulp could be noticed, while in others there was no hyperemia. As a whole, the spleen showed the familiar picture met with in acute infection, including hyperplasia of the pulp and evidence of proliferation in the follicles (fig. 4). Very numerous giant cells of the bone marrow type were present in most of the cases, scattered through the pulp or forming small groups; yet the number of giant cells was not always in

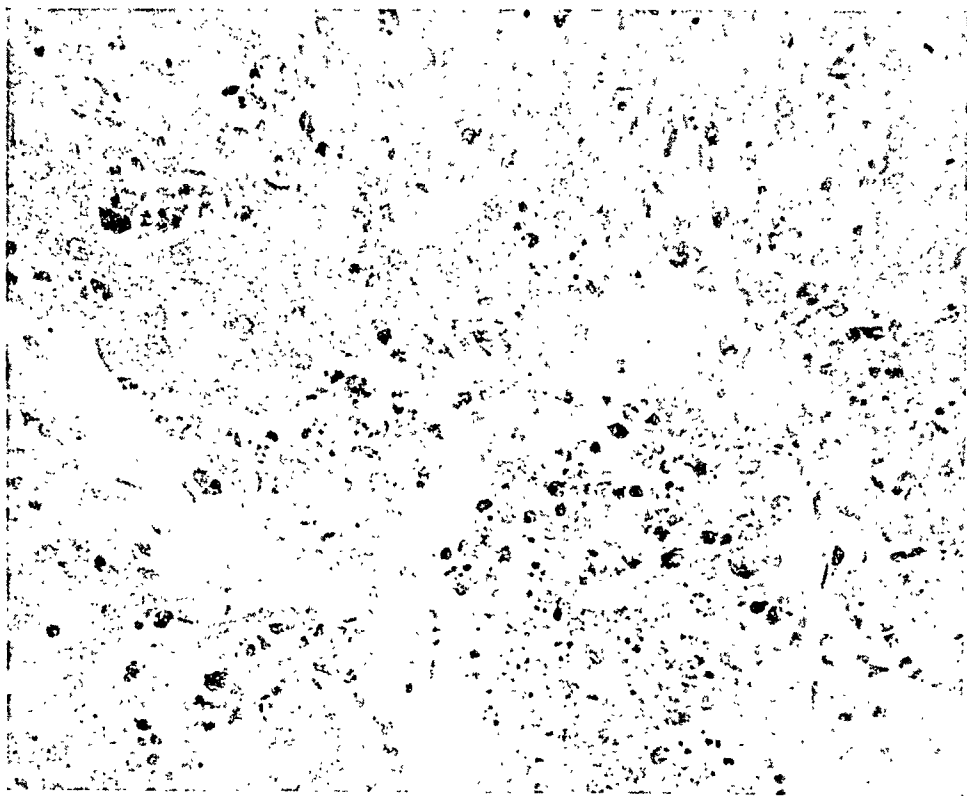


Fig. 2.—Storage of india ink in Kupffer cells of the liver. Note the large size of the desquamated Kupffer cells. The lumen of the large hepatic vein shows many phagocytes loaded with ink.

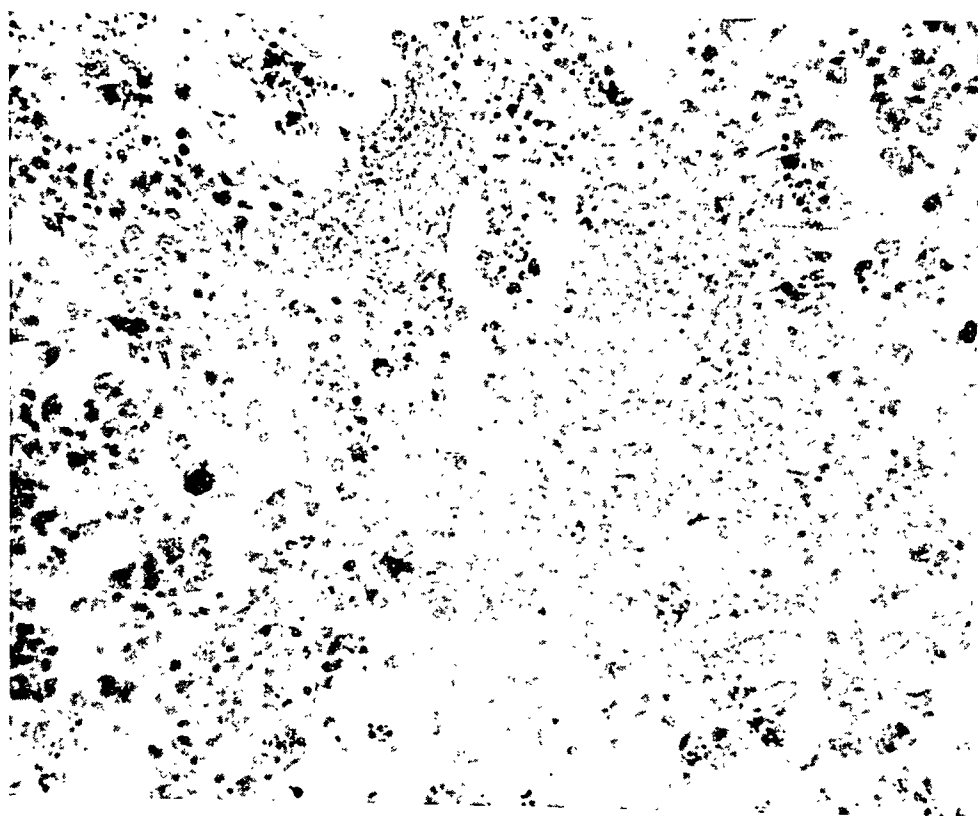


Fig. 3.—Extensive necrosis of the bone marrow. Note the deposits of india ink in the reticulum cells.

excess of what can be seen in the supposedly normal spleen. It seems important to emphasize that the storage of india ink in the spleen fell very far short of that in the other organs, noticeably of that in the liver. The black pigment occurred in the reticulum cells of the pulp and occasionally in those of the follicles (fig. 5). These pigmented cells were scarce, and they contained much less pigment than the Kupffer cells of the same animal.

Phagocytosis of red cells in the spleen was extensive. It was observed chiefly in the pulp. Hemosiderin was abundant in the spleen and was

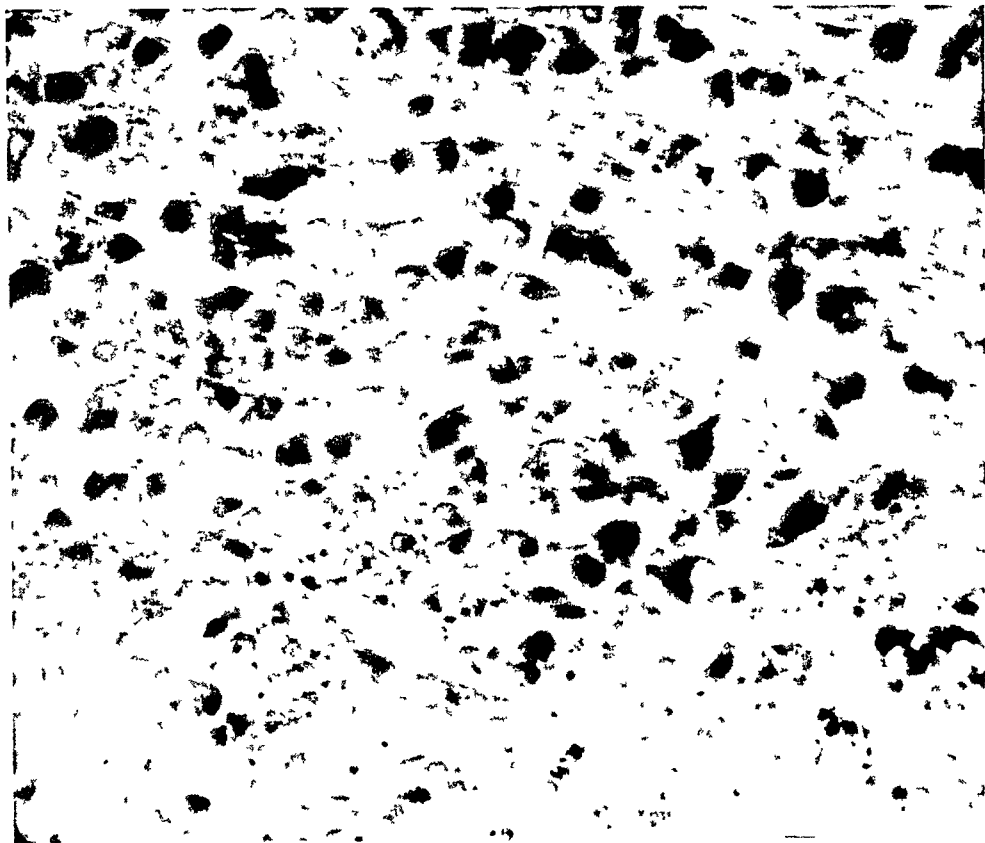


Fig 4—Lymphoblastic plasma cells in the periphery of the splenic follicle.

practically restricted to the splenic pulp. Some of the wider blood vessels contained a large number of cells, many of which were loaded with pigment, while others had ingested red cells.

#### EFFECTS OF BLOCKADE AND SUPERIMPOSED BARTONELLA INFECTION ON SPLENECTOMIZED DOGS AND GUINEA-PIGS

It is generally known that splenectomized dogs and guinea-pigs are not susceptible to the virus of rat anemia. In our experiments, we combined the removal of the spleen with blockade and repeated injections of infectious bartonella material.

Two young dogs were splenectomized. Within the next forty-three days, each received a total quantity of 15 cc. of a 50 per cent india ink solution intravenously and 30 cc. of a 10 per cent solution intraperitoneally, with four intraperitoneal injections of 6 cc. of an emulsion of blood, heart and liver of rats having severe bartonella infection. No bartonellas were ever noted.

Two guinea-pigs were splenectomized, and each was given within the next seventy-two days thirteen intraperitoneal injections totaling 65 cc. of a 10 per cent solution of india ink, with five intraperitoneal injections

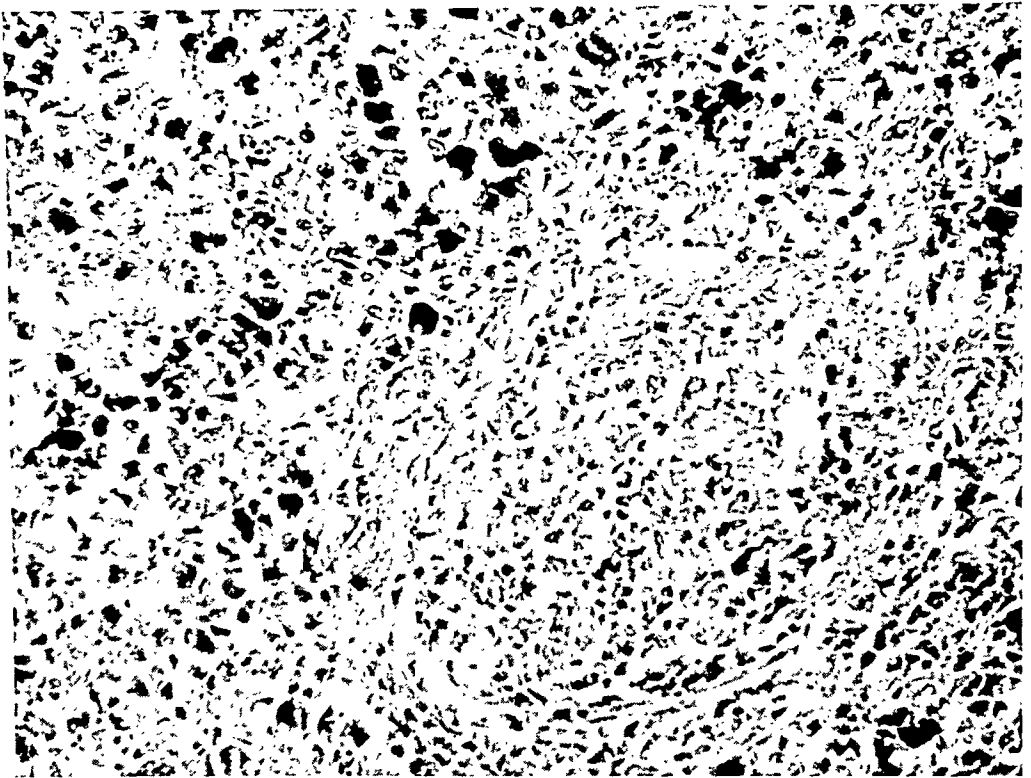


Fig. 5.—Storage of ink in the pulp of the spleen. Note that the large follicles are left free.

aggregating 16 cc. of an emulsion containing abundant bartonellas. Stippling and normoblasts were noted frequently; at times, a few bartonellas were seen.

#### CULTURES FROM BLOCKADED, SPLENECTOMIZED RATS

Blood of rats at the height of bartonella infection was inoculated into ascitic agar, chocolate agar, blood agar, broth and peptone water. In four instances, we obtained a slight growth on blood agar. Microscopically, minute bodies were seen. Subsequent subcultures were, however, negative. In order to study the pathogenicity of this culture, we used a strain of rats that did not originally have a latent bartonella infec-

tion and could therefore be splenectomized without developing bartonella infection and anemia. The injection of the culture into such splenectomized rats failed to give positive results; while control rats of the same strain after injection of an emulsion of blood and liver from bartonella-infected, splenectomized rats, always presented severe anemia and bartonella invasion of the red cells. We were able to obtain a hemolytic streptococcus in cultures from two nonsplenectomized rats that had been blockaded and then infected, and also in cultures from two that had been splenectomized. We obtained a bacillus of the paratyphoid group in cultures from two rats that had been splenectomized and from one that had been blockaded. Thus, the secondary microbes in the case of the splenectomized animals were identical with those in the case of the blockaded, infected animals.

#### CONCLUSIONS

Blockade of the reticulo-endothelial system, combined with a superimposed bartonella infection, produces the blood picture of anemia and a severe bartonella infection. In trypanosome-infected animals, blockade without superimposed infection or superimposed infection without blockade produces the same results.

The cellular changes in the spleen of the blockaded, infected rat consist, essentially, of hyperplasia of the pulp with proliferation of the follicles. In the liver and bone marrow, foci of necrosis are present, irregularly scattered and of different size.

In rats that previously have been splenectomized, have developed a bartonella infection and anemia and have recovered, blockade with india ink or injection of trypanosome-infected blood causes a recurrence of the disease.

# THE NORMAL RENAL GLOMERULUS OF MAN

## HISTOLOGIC CONSIDERATION \*

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Recent anatomic and physiologic studies of the renal glomerulus have led to much new information concerning this structure, but there has been lack of coordination of the knowledge in these two separate fields. This failure of coordination has been due chiefly to the fact that the physiologist has concerned himself almost exclusively with the glomerulus of the kidney of the amphibian, whereas the morphologist has, until recently, given his attention chiefly to the kidney of man. Consideration of the comparative anatomy and physiology of the vertebrate kidney has been lacking until the recent extensive work of Marshall <sup>1</sup> and his collaborators. It has seemed justifiable, therefore, to present the facts of glomerular structure again because of their importance in the light of modern conceptions of glomerular activity, which have been so brilliantly expounded by Richards <sup>2</sup> and his school, and, in addition, in the light of a consideration of anatomic changes that occur in renal disease. It is of great importance to determine definitely the structure of the normal glomerulus of man, since on this knowledge must eventually rest the interpretation of physiologic activity, as well as the understanding of the pathologic changes that may occur in disease. In this study, special attention has been paid to the histology of the normal renal glomerulus.

## REVIEW OF THE LITERATURE

The literature on the structure of the renal glomerulus is of considerable volume, and since it has been carefully reviewed recently by McGregor,<sup>3</sup> no attempt will be made to reconsider the many older studies. The recent application to the kidney of the stain combining Mallory's aniline blue and Heidenhain's azan carmine, as described by McGregor, has opened an entirely new approach to the study of the structure of the normal glomerulus, and it has brought out some important considerations, not only of normal glomerular structure, but of the pathogenesis of glomerular disease.

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\* From the Section on Pathologic Anatomy, the Mayo Clinic.

1. Marshall, E. K., Jr.: *Am. J. Physiol.* **94**:1, 1930.

2. Richards, A. N.: *Pennsylvania M. J.* **33**:527, 1930.

3. McGregor, Leone: *Am. J. Path.* **5**:545 and 559, 1929.

There is still controversy, however, concerning some details of glomerular structure among those who have used modern staining methods, and since it seems important to settle these points, they will be outlined.

The normal human renal glomerulus is composed of a group of intertwining capillary loops, which probably do not anastomose, which are formed by division of the afferent glomerular arteriole and which rejoin to form the efferent glomerular arteriole. These loops are covered by a layer of epithelium which comprises the visceral layer of Bowman's capsule, and these cells are directly continuous, therefore, with the epithelial cells of the tubules. The average diameter of the human glomerulus in fixed tissue is estimated by Vimtrup<sup>4</sup> as 200 microns, by Fahr<sup>5</sup> as 237 microns, whereas, in the living frog, Richards and Schmidt<sup>6</sup> found the diameter to be from 140 to 300 microns. Russell<sup>7</sup> reported the average glomerular diameter in frozen sections of human kidneys to be 118 microns.

The component parts of the glomerulus are: (1) the capillary or glomerular endothelial cells; (2) the glomerular membrane between the cell layers; (3) the epithelial cells overlying the loops, and (4) occasional connective tissue cells and fibers. Although these components have been recognized for a long time, there has been considerable difference of opinion as to their exact relationship to one another, as well as to the correct derivation of each. With the application of newer methods of staining within the last few years, this problem has apparently become simplified. The use of the aforementioned stain, combining Mallory's aniline blue and Heidenhain's azan carmine, has aided greatly in the determination of the structure of the normal glomerulus, and for the first time an adequate method of differentiating epithelial and endothelial cells in the glomerular tuft has been found. In addition, the first detailed description of the glomerular basement membrane has been accomplished. These two factors become of considerable significance now in the interpretation of glomeruli as normal or abnormal and in determining and evaluating glomerular changes in disease.

Recently advanced interpretations of glomerular histology indicate that the afferent glomerular arteriole, which is composed of a muscular media (circular fibers) and an elastic interna, pierces almost to the center of the loops of the interwoven capillaries before breaking up into

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4. Vimtrup, B. J.: *Am. J. Anat.* **41**:123, 1928.

5. Fahr, T., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1925, vol. 6, pp. 6-290.

6. Richards, A. N., and Schmidt, C. F.: *Am. J. Physiol.* **71**:178, 1924.

7. Russell, Dorothy S.: Medical Research Council, Special Report Series, no. 42, 1929.

from two to four, or at times more, primary branches, which in turn break up into the secondary branches or capillaries. After forming a series of interlacing, but apparently not anastomosing, loops these secondary branches rejoin to form the efferent arteriole, or vas efferens, composed of smooth muscle (circular fibers) without any elastic membrane (Maximow<sup>8</sup>) or composed of a circular network of cells (pericytes of Zimmerman) without muscle fibers (Bensley<sup>9</sup>). Each individual capillary loop is closely covered when not in contact with another loop, by an epithelial investment composing the visceral layer of Bowman's capsule. The afferent and efferent vessels are supplied by nerve fibers, but it is questioned that the capillary loops themselves receive any direct or indirect nerve supply. It has generally been considered that the diameter of the vas afferens is twice that of the vas efferens, but recent work by Bensley would seem to question this definitely, and he has expressed the belief that they are of approximately equal size.

In the average section of from 10 to 15 microns in thickness, made through the greatest diameter of normal glomeruli, the total number of cells present, according to Mertz,<sup>10</sup> is constant between 120 and 141. He found from 3 to 30 of these to be leukocytes, and the remainder he did not differentiate. Von Möllendorff<sup>11</sup> and Bargmann<sup>12</sup> both concluded that there were at least ten times as many epithelial as endothelial cells within the tuft, although quantitative studies were not reported. McGregor concluded that endothelial cells were much less numerous than epithelial cells.

The presence of endothelial cells within the capillary loops of the glomerulus has long been conceded, but the absolute number present, and the presence or absence of boundaries between them, have been disputed. Von Möllendorff described the endothelium of the glomerulus in man as consisting of a fine layer with few nuclei, and with visible cytoplasm only at points where endothelial nuclei were found. He did not observe cell boundaries between them.

Russell observed that "the capillaries are lined with an apparently interrupted layer of endothelial cells." In addition to the endothelial cells within the capillaries McGregor described certain cells "probably endothelial, which lie inside the glomerular basement membrane, but partially surrounded by it."

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8. Maximow, A. A.: *A Textbook of Histology*, ed. 1, Philadelphia, W. B. Saunders Company, 1930.

9. Bensley, R. D.: *Am. J. Anat.* **44**:141, 1929.

10. Mertz, Albrecht: *Centralbl. f. allg. Path. u. path. Anat.* **29**:321, 1918.

11. von Möllendorff, Wilhelm: *Ztschr. f. Zellforsch. u. mikr. Anat.* **6**:441, 1927.

12. Bargmann, W.: *Ztschr. f. Zellforsch. u. mikr. Anat.* **8**:765, 1929.



Exact studies of the glomerular basement membrane have been presented only recently, since they have depended on the development of an adequate technic of staining. Various descriptions of this membrane have been given. Von Möllendorff described the membrane as a true structure in the glomerulus, homogeneous and formed by the endothelium. In addition, he expressed the belief that the cytoplasm of the overlying cells sent out long processes, mostly parallel with the long axis of the capillaries, and that these processes divided into finer branches. Volterra,<sup>13</sup> however, described the membrane as a reticular adventitia, in the form of a continuous membrane of netlike type, in which the network consisted of argyrophil connective tissue fibrils. He interpreted the cells overlying it, that is, "deck" cells, as being pericytes adherent to the membrane and forming it. The endothelium simply rested on the membrane. Corner<sup>14</sup> had also previously expressed his view that "the endothelium of the glomerular tuft is in no case provided with reticulum, affording a marked contrast to that of the intertubular capillaries."

Bargmann described the glomerular membrane as a deep black or brown granular line when impregnated with silver. Russell observed the membrane to be a "delicate refractile basement membrane." McGregor found the membrane to be homogeneous for the most part, although near the hilus a laminated appearance occurred at times. "Whether the membrane is fibrillar or whether the extra fibrils belong to the connective tissue cells of the vascular pedicle has not been determined. . . . (It) does not stain with any of the silver impregnation methods used." The origin and nature of the basement membrane have been disputed. As noted previously, von Möllendorff expressed the belief that it is a homogeneous, structureless membrane of endothelial origin. To this Bargmann agreed. Volterra, however, believed it to be a connective tissue structure formed by the pericytes or deck cells surrounding the loops, since it acts as a connective tissue structure in taking the silver impregnation and also Mallory's stain. He also expressed the belief that the processes which von Möllendorff described were really connective tissue fibrils that formed a histologic unit with the basement membrane, and that instead of being of variable thickness, the fibrils were uniform in width. The lack of uniformity of opinion as to the embryology of the glomerulus prevents a definite statement as to the origin of the membrane. McGregor noted, however, that the membrane was "closely associated with the glomerular epithelium and was present before the glomerular capillaries appeared." Although the glomerular membrane is directly continuous with the

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13. Volterra, M.: *Ztschr. f. Zellforsch. u. mikr. Anat.* **7**:135, 1928.

14. Corner, G. W.: *Carnegie Inst., Contrib. Embryol.*, 1920, vol. 9, p. 87.

basement membrane of the tubules, it is not similar physiologically, as will be pointed out later. The questions which are unsettled in regard to the normal membrane are therefore: (1) its origin; (2) the presence or absence of a fibrillar structure, and (3) the presence or absence of the property of being impregnable with silver.

Discussion of the glomerular epithelium or "deck Zellen" raises the question as to the exact nature of these cells. They have been called pericytes, adventitial cells and epithelial cells. Some workers have considered them to be vascular cells; others believe them to be epithelial cells. Von Möllendorff described them as being morphologically similar to the adventitial cells or pericytes of the capillary bed. He described the long, branching processes that have been noted. Volterra expressed his belief that the cells are perithelial, but he did not see any processes on them. Bargmann agreed with von Möllendorff as to the presence of processes in the deck cells. Russell stated that the epithelial cells covering the loops frequently "appear to be absent from the convexities of the lobules though small groups can be detected within the interlobular clefts. In some kidneys a complete investment of the tuft has been seen, these kidneys were pathological, but there is no reason to believe that the pathological changes did more than render the normal structure more prominent." McGregor remarked: "These cells form a complete single layer covering over the tips and crevices of the capillary tufts. At the hilum they are continuous with the capsular, and thus with the tubular, epithelium. They lie outside the glomerular basement membrane which separate them from the endothelium. The arrangement is not syncytial and cell boundaries are easily seen. They are much more numerous than endothelium."

The presence of connective tissue cells within the tuft is generally believed to be limited to the hilar portion of the glomerulus. Undoubted connective tissue cells and their fibrils are to be seen in this portion of the tuft, and they are well shown by the use of silver stains. Although older writers believed them to be present in the periphery of the tuft, recent careful observation has failed to sustain this impression.

The glomerulus has been considered to be a structure for the filtration or secretion of the glomerular fluid from the blood plasma. It is not my purpose in this paper to enter into a detailed consideration of the various theories of glomerular function. The brilliant studies of Richards and his collaborators,<sup>15</sup> have given ample evidence to warrant the assumption, and more recently they have conclusively proved, that filtration occurs in the glomerulus of the amphibian kidney. It is not necessary to elucidate this point, the details of which are familiar to physiologists and clinicians as well. However, in the recent work of

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15. Richards, A. N.: *Am. J. M. Sc.* **170**:781, 1925; footnotes 2 and 6.

White, evidence has been presented again that might lead one to believe that glomerular secretion was to be considered as a factor in the elaboration of this fluid. It might also be stated that there is still a feeling among certain workers that glomerular secretion does exist. This is exemplified by the work of White<sup>16</sup> that has been noted, as well as by the opinion expressed in a recent textbook: "One conclusion can be safely deduced from the study of the structure of the kidney. The malpighian corpuscle with its glomerulus and capsule of Bowman must act as a filtration apparatus." And yet the author adds: "Thus it is possible that the passage of the liquid through the endothelium of the glomerular capillary tuft, and through the covering epithelium, may be at least partly 'a vital phenomenon.'"

#### MATERIAL AND METHODS OF PRESENT STUDY

The present study was made on a series of kidneys obtained post mortem from subjects who were apparently in normal health until the time of death. The group included young persons only, all less than 40 years of age, who were killed accidentally or who died shortly after injury. Postmortem material was studied because it is the type of renal tissue generally observed by the pathologist, and an understanding of the appearance of normal postmortem renal substances is essential to the proper interpretation of renal tissue as normal or abnormal.

The kidneys of twenty-five, apparently previously healthy, subjects were studied. The tissues, which were obtained from one to four hours post mortem, were fixed in Zenker's fluid or in formaldehyde 10 per cent. Tissues fixed directly in Zenker's fluid gave the more satisfactory results. Tissues fixed in formaldehyde were taken to Zenker's fluid by the method of Davidoff<sup>17</sup> ammonia water, which was fairly satisfactory, and by the use of the Weigert mordant,<sup>18</sup> which gave much more satisfactory results. The latter method had the slight disadvantage of being more time-consuming. Paraffin sections were cut at a thickness of 8 microns, and the following stains were used: hematoxylin and eosin, van Gieson's sudan III, Orlandi's (Bielschowsky's modification by Noel) and Heidenhain's azan carmine modification of Mallory's aniline blue.

There are several minor disadvantages in the latter method of staining. Uniform results are not always obtainable, although tissues may be uniformly handled. This necessitates at times individualization of sections, which, however, is not difficult. Another difficulty is that the variability of absorption of the azan carmine portion of the stain by erythrocytes and fixed cells is such that even within the same section differentiation of cells is unsatisfactory on the basis of staining reaction alone, and one must, therefore, depend on the morphologic differences and the position of the cells in relation to other known structures, for example, the basement membrane.

16. White, H. L.: *Am. J. Physiol.* **90**:689, 1929.

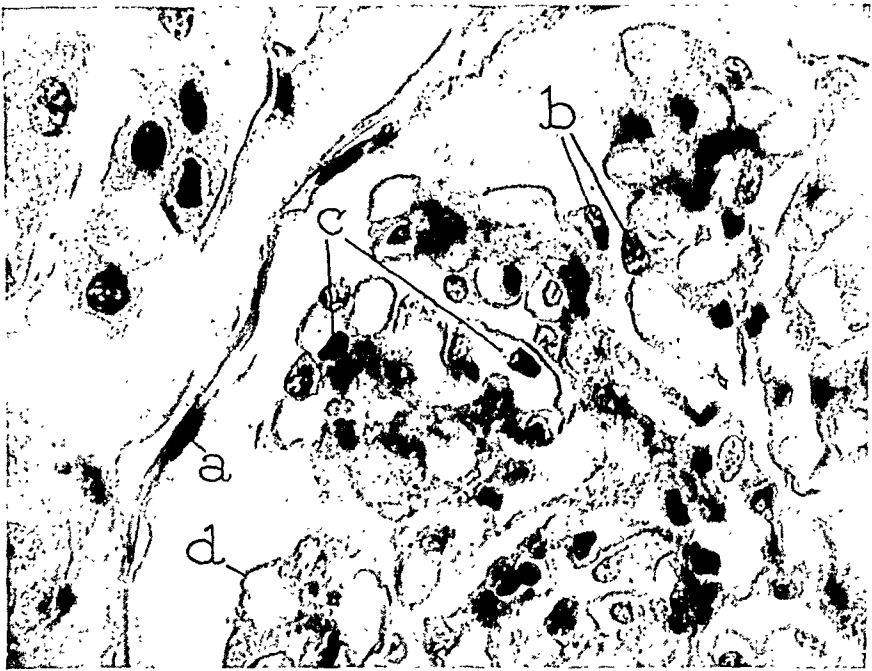
17. Davidoff, L. M.: *Am. J. Path.* **4**:493, 1928.

18. Kernohan, J. W.: Personal communication to the author.

## OBSERVATIONS

In general, the appearance of the glomeruli studied is similar to that described by McGregor, but several important deviations are to be pointed out (fig. 1).

*Glomerulus as a Whole.*—Glomeruli in the same microscopic section from a normal kidney show considerable variation in general appearance. There is variability as to size of the tufts, as to the presence or absence of large numbers of erythrocytes and as to the number of capillary loops that are wide open and dilated. Certain glomeruli appear large and wide open, with capillaries that may or may not be filled with



Portion of a normal human renal glomerulus; *a*, capsular epithelium; *b*, glomerular epithelium; *c*, endothelium, and *d*, glomerular membrane. Many of the epithelial cells have been desquamated;  $\times 720$ .

erythrocytes; other glomeruli, cut apparently through their greatest diameter, are much smaller, bloodless, and with little, if any, capillary space visible. With the use of ordinary stains such glomeruli might be considered abnormal, but with the use of the Mallory-Heidenhain stain (aniline blue and azan carmine), which brings out the individual features of the glomeruli, proper interpretation can be made.

The afferent arteriole, when it is visible, is often very large, and appears much larger in diameter than the efferent arteriole, which is less frequently seen.

Occasionally glomeruli with partial or complete hyalinization are observed in the normal kidney.

*Glomerular Epithelium.*—The glomerular epithelium forms a single, continuous layer of squamous epithelial cells, with cell boundaries, overlying the capillary loops of the tuft, and continuous with the epithelium that lines the parietal wall of Bowman's space. The cytoplasm is usually a thin layer, except in the region of the nucleus, which is usually elongated. In sections of kidney, as they are generally obtained post mortem, a considerable portion of both the visceral and the parietal layer of epithelium of Bowman's capsule may be desquamated. These cells are the first in the glomerulus to desquamate post mortem, and improper interpretations are easily made, especially if many of the capillary loops are bare. These desquamated cells are frequently the source of a considerable amount of the débris that is commonly found within the capsular space. This débris may erroneously be interpreted as albuminous material coming from the capillary loops. Its rather coarse granular appearance, however, distinguishes it from the more homogeneous glassy appearance of coagulated albumin. The epithelial cells are the least important of the glomerular structures in the study of glomerular changes in disease.

*Glomerular Endothelium.*—The glomerular endothelium is visible as an apparently interrupted layer of flattened nuclei surrounded by a minute amount of cytoplasm within the glomerular membrane. There are several features worthy of attention. In the first place, although cytoplasmic bridges between the individual nuclei are not ordinarily visible, they are occasionally seen when the nucleus along with the cytoplasm is detached from the underlying membrane. In such an instance the cytoplasm can be observed as a thin line, frequently several times the length of the nucleus, extending in both directions from it. Cell boundaries have not been observed. Another feature of importance is the frequent occurrence of endothelial cells, which appear large and swollen, but not vacuolated, and which project for a considerable distance into the lumen of the capillary and would therefore seem partially to obstruct it. These cells are so frequently observed that they cannot be considered to be abnormal. In a large, dilated, blood-filled capillary loop the endothelial cells are flattened; whereas, in a small capillary loop that appears somewhat contracted and is usually bloodless or contains an occasional erythrocyte only, the endothelial nuclei are frequently more oval or rounded, and they seem to occupy relatively more space within the capillary. Evidences of proliferation and hyaline fibers have not been observed under such conditions.

It is also to be pointed out that the number of endothelial cells in relation to the number of epithelial cells is variable. Desquamation of epithelial cells, which is a normal postmortem occurrence, is likely to render erroneous relative counts of the numbers of endothelial and of epithelial cells in the normal person. Unless a given glomerulus is

well covered with epithelial cells and unless desquamation is absent, counts are of little value. In the present study, numerous counts of the normal number of cells have been made, and from this experience estimates have been made relative to other glomeruli, without quantitative examination. These studies show that in the ordinary glomerulus from one fourth to one sixth of all nuclei observed, exclusive of those of leukocytes, are of endothelial cells.

Nuclei are found frequently within the glomerular membrane at points other than the hilus, and they have the appearance of endothelial nuclei. Cytoplasm surrounding them is rarely visible. These cells may be significant in the origin of the membrane, and they occasionally are abundant.

*Glomerular (Basement) Membrane.*—The basement membrane of the glomerulus is an important part of the glomerulus, forming its basic structure, and it undergoes marked changes in nephritis and hypertension. These changes, which are in large part characteristic of the disease present, are interpreted correctly only if the appearance of the normal glomerular membrane is known. In the normal glomerulus presenting widely dilated loops, the membrane is a thin, blue, homogeneous line with the Mallory stain; in loops that are not so widely dilated, the membrane is thicker, may have a fibrillar appearance, and not infrequently contains nuclei within it. The membrane is thickened not only at points where the capillary loops divide, but also occasionally at points where branching cannot be observed. These thickened portions usually do not decolorize as readily as the thinner portions of the membrane. Inadequate decolorization of the glomerular membrane (stained with Mallory's aniline blue) may lead to appearances suggesting a thickened membrane, an appearance which further decolorization will obliterate.

Studies of the glomerular membrane have been made with the Orlandi silver impregnation method, but the membrane did not become impregnated with silver in any of the cases observed. The outer layers of the capsular membrane, however, do take up the silver, for they are part of the intertubular structural framework, which, of course, becomes impregnated with silver also.

*Connective Tissue.*—Connective tissue cells and fibrils are always to be seen at the hilus of the tuft, and they may extend as far as the central portion of the glomerulus. The nuclei, which resemble those of the endothelial cells, frequently can be distinguished by ordinary stains, since they are more elongated and less oval. At times these cells are numerous at the region of the hilus. With silver impregnation methods, these cells and their fibrils stand out distinctly.

*Leukocytes.*—Leukocytes, usually polymorphonuclear, are to be seen scattered throughout the capillary loops. The average glomerulus rarely contains more than fifteen such cells. Any type of leukocyte observed in the blood may be seen within the glomerular capillary loops.

*Tubules, Blood Vessels and Other Structures.*—Little attention has been paid to the tubules in the present study because of the variability of their staining reactions with the Mallory-Heidenhain stain. Uniformity is lacking, and nothing distinctive that cannot be observed by the simpler stains has been observed. Hyaline droplets within the tubular cells take a stain varying from bright red to orange. The inter-tubular network is stained a bright blue by this method. The erythrocytes within the capillaries show variation of color from gray to yellow, orange or red, even within the same capillary loop. The structure of the smaller and of the large blood vessels is admirably shown by the use of this stain.

#### COMMENT

*Anatomic Considerations.*—The Mallory-Heidenhain stain has proved to be invaluable in the study of the renal glomerulus and is worthy of use as a routine in the study of postmortem material in the pathologic laboratory. Its chief advantage is that it stains the component parts of the glomerulus so that they can be distinguished from one another and can be studied separately. This allows a much more careful analysis of glomerular changes as they are seen under the microscope. It is important to point out, however, that before interpretation of the abnormal is made, careful study and understanding of the normal glomerular structure is necessary.

Several points have been mentioned that are believed to be of significance in the consideration of the structure of the normal glomerulus of man. These are: (1) the variability in size of the capillary loops and the consequent variability in the thickness of the glomerular membrane; (2) the presence of regions of thickening in the membrane at points other than the branching of capillaries, and the fibrillar appearance of the membrane at such points of thickening; (3) the number and characteristics of the epithelial and endothelial cells of the tuft; (4) the relatively large number of cells found within the glomerular membrane; (5) the relative infrequency of leukocytes, and (6) the failure of the glomerular membrane to become impregnated with silver.

The glomerular membrane has become the most distinctive histologic feature of the glomerulus since the application of this method of staining. Interpretations of its structure and appearance vary and it must be taken into consideration in physiologic interpretations, as has been mentioned. It plays an important part in the pathogenesis of glomerular

disease. The nature of this membrane and its relationship to the other basement membranes of the kidney is uncertain, as will be brought out.

The presence of numerous thickened portions in the normal glomerular membrane is of significance, for this might readily be considered to be abnormal. These thickened portions occur at junctures of capillaries, as pointed out by McGregor, and they are also seen where capillary branching does not occur. Improperly decolorized regions must not be confused. In such thickened portions the membrane has a fine fibrillar appearance, and endothelial nuclei are not infrequently found within the membrane at such points. No further light has been thrown on the origin of the membrane in the present study. The nuclei found within the membrane may possibly be of some significance in this respect. Thickening of the glomerular membrane occurs in cases of glomerular nephritis and hypertension and has been reported in cases of lipoid nephrosis,<sup>19</sup> and although in most of these cases the thickening is obvious, in others it has been found to approach the normal appearance. The failure of the glomerular membrane to become impregnated with silver is noteworthy. Although the glomerular membrane is directly continuous with the intertubular and capsular membranes which become impregnated with silver, the glomerular membrane does not act similarly. This is a distinguishing factor which cannot be overlooked. Although it cannot be adequately explained at present, it signifies a difference in chemical structure between this membrane, which is apparently exclusively a filter, and the intertubular membrane of the kidney. Whether or not there is a difference in the function of these two types of membrane, which are apparently continuous, remains to be determined. Corner studied the reticulum in various organs, especially the kidney, and he came to the conclusion: "In the renal cortex, between the convoluted tubules and about the glomerulus, the 'stroma' is no more nor less than a network of reticular fibrils embedded in the cytoplasm of the capillary endothelium or deposited by the latter against the tubules and Bowman's capsule. True fibroblasts are very infrequent—perhaps altogether absent." This conception that fibers of reticulum are produced by endothelial cells was not new, since Mall<sup>20</sup> and Evans<sup>21</sup> previously had reported such a structure in other organs; for example, the suprarenal glands, the thyroid gland and corpus luteum. Corner continued: "The endothelium of the glomerular tuft is in no case provided with reticulum, affording a marked contrast to that of the intertubular capillaries." That there may be differences between capillary endothelial cells within the glomerulus and the intertubular blood vessels seems possible; Corner stated: "It seems, therefore, that capillary

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19. Bell, E. T.: *Am. J. Path.* **5**:587, 1929.

20. Mall, F. P.: *Math. Phys. Abhandl.* **17**:295, 1891.

21. Evans, H. M., quoted by Corner (footnote 14).



endothelium can form reticulum fibrils in those organs whose blood capillaries come into direct contact with active secreting epithelial cells. Where support of tissues is by connective tissue, the endothelium seems devoid of reticulum fibrils. It will be important to determine if these two types of fibers are chemically different and if the endothelial cells which form and those which do not form fibers are different." There are two other points of difference between the glomerular membrane and the tubular basement membrane which are worthy of mention. The first is that the glomerular membrane decolorizes more rapidly than does the tubular basement membrane during the process of staining with Mallory's aniline blue. The second point is that fibrous tissue change occurs much more readily under abnormal conditions in the intertubular reticulum than within the glomerulus. This is illustrated by the example occasionally seen, in which, along with an increase in the fibrous tissue between the tubules, there is also an increase of this tissue surrounding the parietal layer of Bowman's capsule, at times compressing it, and at times with no change within the glomerular tuft itself. Careful study of such glomeruli reveals that the proliferation of fibrous tissue which has occurred around the glomerular capsule involves the outer layer of the double covering, which is in fact made up of the intertubular reticulum. The inner layer, which is a homogeneous, narrow membrane that does not become impregnated with silver, is not a part of the process, and there is no change in the glomerulus. It is a possibility that the intracapillary hyaline fibers formed between the proliferating endothelial cells in the glomerular tuft in glomerular nephritis may be endothelial in origin, since these fibers occasionally become impregnated with silver, as do the reticular fibrils in the intertubular regions. Since all the cells within the glomerulus are of mesenchymal origin, it is theoretically possible that any type of cell, particularly under abnormal influences, may produce fibrils. The fibrils found in the crescent, which have a similar origin, become impregnated with silver also.

Attention should be called to the distinguishing features of the epithelial and endothelial cells of the tuft. Although certain minor morphologic and staining differences exist between these cells, the most accurate method of differentiating them is by determining their relationship to the glomerular membrane. All nuclei, excluding those of leukocytes, inside the glomerular membrane are endothelial, and all nuclei outside it are epithelial, regardless of form or staining reaction. The position of the cell in regard to the membrane is by far the most distinguishing feature and the only one that should be used at present.

Recent reports in the literature indicate that glomerular epithelial cells are ten times as abundant as glomerular endothelial cells. The present study has not borne out this statement, since it has been found

that approximately from one fourth to one sixth of all glomerular cells, exclusive of leukocytes, are endothelial. This may be of importance in the consideration of early changes in glomerulitis, since endothelial proliferation is probably the first important step in this process. Endothelial proliferation has been reported in cases of clinical glomerular nephritis and in lipoid nephrosis. As in thickening of the glomerular membrane, marked proliferation of endothelial cells is easily discernible, but a slight amount of proliferation and increase in number of cells may be confused with the normal. It should be mentioned, also, that the endothelial cells which normally appear rounded and oval, and which partially obstruct small capillary loops, may be mistakenly identified as proliferating endothelial cells. In addition, the large number of endothelial cells in the normal condition makes it more reasonable to believe that the capillary wall is completely covered by a layer of endothelial cells and not by an apparently interrupted layer of endothelial cells, as recorded by Russell. Boundaries between the endothelial cells have not been observed.

The presence of endothelial cells within the membrane is more difficult to explain. These cells may have some significance from the embryologic standpoint, they may be of significance in the formation and maintenance of the membrane in the adult glomerulus, or they may possibly be the remnants of previous capillary loops that have collapsed and disappeared, leaving the lining endothelium within the layers of the glomerular membrane, which in time becomes thinner. Since processes or fibers within these cells cannot be seen, it is difficult to interpret them exactly.

The cells covering the glomerular tuft, over which there has been considerable discussion, as already mentioned, are most probably epithelial cells and not vascular or adventitial cells or pericytes. They are, most likely, epithelial, because they cover a surface, and because they are directly continuous with, and react similarly and synchronously with, the tubular epithelial cells in desquamation and degeneration. In addition the glomerulus of the human infant presents epithelial cells that are columnar or cuboidal and closely compacted, an appearance that strongly suggests that they are epithelial cells. It is only as the adult stage is reached that these cuboidal cells flatten out and give the squamous appearance usually observed. Processes arising within these cells and extending beyond them have not been observed. Russell stated that the epithelial cells covering the loops frequently "appear to be absent from the convexities of the lobules." She wrote, also, "In some kidneys a complete investment of the tuft has been seen; these kidneys were pathological, but there is no reason to believe that the pathological changes did more than render the normal structure more prominent."

It seems obvious that such statements are the result of observing normal glomeruli from which the most of the epithelium had been desquamated.

The presence of leukocytes within the capillary loops is not of particular significance. In the present study a smaller number has been found than has been previously reported. It was formerly believed by some that the proliferation of cells within the glomerular capillaries in nephritis was of large mononuclear leukocytes. This does not seem likely at present, since careful study has shown these proliferating cells to be the endothelial cells of the capillary walls. In certain severe infections, such as pneumonia, one occasionally sees large collections of leukocytes (mononuclear and polymorphonuclear) in the glomerular capillaries, but the reaction is different from that seen in glomerulitis in clinical glomerular nephritis. Leukocytes are also to be found in the glomerulus in suppurative or embolic glomerular disease. In the normal glomerulus all types of leukocytes are found, just as they occur in the circulating blood.

*Physiologic Considerations.*—The anatomic evidence, considered in the light of present physiologic knowledge, seems as overwhelmingly in favor of a filtration theory of glomerular function as does the physiologic evidence. This conclusion is adduced following careful study of the normal glomerular structure in man. The glomerulus anatomically seems admirably adapted for the physiologic process of filtration and, in addition, does not contain any structure that seems adapted for secretion as this term is ordinarily used. The structures concerned are several: (1) endothelial cells, which apparently are the same as capillary endothelium elsewhere in the body, and therefore should not be considered as secretory cells; (2) the glomerular membrane, which obviously cannot secrete, and (3) the epithelium, the cells of which are the only structures in the glomerulus which might possibly secrete, but which, although directly continuous with, and perhaps derived with, the tubular epithelium from the same embryonal tissue, careful study and thought indicates are not secretory cells. The paucity of cytoplasm in these cells, their close resemblance to the parietal epithelial cells of Bowman's capsule and other squamous cells, and their relatively small number, considering the large volume of glomerular fluid produced, are anatomic points strongly in favor of the absence of secretion. It should be mentioned that the failure of the glomerulus to secrete does not class it as a "dead membrane," as it is sometimes referred to, any more than does the failure of the capillary membranes elsewhere to secrete classify them similarly. It might also be noted that there is some similarity between the glomerular wall and the wall separating the narrow descending limb of Henle's loop from the intertubular capillary surrounding it. That is, these walls are made up of narrow, pavement epithelium, a

basement membrane and endothelial cells. As far as is known, secretion has never been considered as occurring in this region.

The observation of the variability in size of the glomerular capillary loops in renal tissue of man, obtained after death, may be the result, in part, of the physiologic state of the tissue at the time of death. As has been mentioned, the glomerular capillary loops may appear large and dilated, and filled or empty of blood, or they may be small, with a lumen that would hardly admit a single erythrocyte. All variations between these two states are seen in normal glomeruli in the same or different kidneys. Corresponding with these states, one may find the glomerular membrane thin, or thickened with endothelial cells, which may be flattened and without visible cytoplasm or may have nuclei that definitely obstruct the lumen of the loop. The observations of Richards and Schmidt on the kidney of the living amphibian led them to the conclusion that the individual capillaries did not vary in caliber, but that intermittence of flow in individual loops depended on the contraction and relaxation at the juncture of the capillary with the afferent and efferent arterioles. The capillary loops, in other words, were passive structures, the flow of blood through which depended on the opening and closing of "sphincters" at the extremities of the capillary loops. However, the distensibility of the capillaries is admitted by Richards;<sup>15</sup> it has been observed by him directly in the frog's kidney and has been indirectly inferred from changes in renal volume following the application of chemical stimuli to the living kidney.

The intermittency of action of the whole and of part of the renal glomerulus of man is indicated by the observations just cited. This observation may prove to be important also from the standpoint of determining the state of the renal circulation at the time of death in such conditions as acute renal insufficiency and shock. It is of value also from the standpoint of interpretation of glomerular changes, such as occlusion of a single capillary loop, or in the absence of erythrocytes from capillary loops, for such appearances might readily be confused with abnormal conditions sometimes seen. The use of this stain (Mallory-Heidenhain) brings out details which either cannot be interpreted or which are misinterpreted when seen in sections stained by ordinary methods.

#### CONCLUSIONS

The anatomic evidence, considered in the light of present physiologic knowledge, seems as overwhelmingly in favor of a filtration theory of glomerular function as does the physiologic evidence.

The Mallory-Heidenhain (aniline blue and azan carmine) stain, which stains the individual structures of the glomerulus distinctively,

is worthy of being used as a routine in the laboratory in the study of renal tissue obtained post mortem.

There is marked variability in the size of the glomerular capillaries observed post mortem in a single kidney, and this may be evidence of the state of the renal circulation at the time of death. It may possibly indicate the intermittence of glomerular activity in the kidney of man.

The glomerular membrane is of variable thickness, is at times fibrillar in appearance, and occasionally contains many nuclei. The chemical properties of the glomerular membrane differ from those of the intertubular membrane of the kidney.

Endothelial cells are more numerous, and leukocytes less numerous, in the glomerulus than has previously been indicated.

# EFFECT OF INJECTION OF TRYPAN BLUE ON RATE OF SEDIMENTATION OF ERYTHRO- CYTES IN INFLAMMATION \*

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Since it is recognized that inflammation usually acts as a protective mechanism, it is natural to attempt to discover common factors in the production of antibodies and in the process of inflammation. It seems to be possible that those cells which many believe to have a rôle in the production of antibodies, the cells of the so-called reticulo-endothelial system, may be involved in the process of inflammation and particularly in the increased production of fibrinogen that usually follows inflammation. This possibility was suggested by an observation by Freund<sup>1</sup> on the influence of age on the production of antibodies and the response to inflammatory irritants: Studying the immunity conditions in adult and in very young animals, he found that the production of antibodies is poor in young rabbits. He also found that the intracutaneous injection of pneumococci causes only a slight rise in the rate of sedimentation of red blood cells in young rabbits as compared with the greater rise that he observed in adult rabbits.<sup>2</sup>

It has been known for a long time that an increase of blood fibrinogen accompanies the infectious diseases associated with leukocytosis, such as pneumonia, erysipelas, scarlet fever, etc.<sup>3</sup> Fahraeus noticed that an increase in the rate of sedimentation of red cells occurred in many diseases; that an increase in the rate is accompanied by an increase of blood fibrinogen; that these changes are fairly parallel. Increased formation of rouleaux is uniformly present in smears made from blood that has a high rate of sedimentation, and this increased formation of rouleaux is due to physical changes in the red cells brought about by a rise in the amount of fibrinogen.<sup>4</sup> The formation of rouleaux binds numbers of red cells into masses, and the sinking velocity of such aggregations becomes greater as their size increases.

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\* From the Department of Pathology, School of Medicine, University of Pennsylvania.

1. Freund, J.: *J. Immunol.* **11**:383, 1926; **18**:315, 1930.

2. Freund, J.: Personal communication to the author.

3. Pfeiffer, T.: *Ztschr. f. klin. Med.* **33**:214, 1879; *Centralbl. f. inn. Med.* **19**:1, 1898.

4. Fahraeus, R.: *Physiol. Rev.* **9**:241, 1929.

In recent years it has been established that the injection of india ink or other dyestuffs, such as trypan blue, carmine, janus green, etc., into guinea-pigs, rabbits, rats and mice causes a retardation or a diminution in the formation of antibodies,<sup>5</sup> also in the development of sensitiveness to tuberculin, and interferes with the chemotherapeutic action of certain drugs. The action of the dyes is frequently interpreted as a "blockade" of those cells in which the presence of the dyestuffs can be demonstrated by histologic examination, i. e., the so-called reticulo-endothelial cells. The experiments of Smith,<sup>6</sup> however, clearly showed that when the administration of certain dyes is followed by the injection of another dye, both dyes can be found in the cells. In the light of these experiments it is possible that the injection of dyes does not "block" the cells to the entrance of antigens. It seems probable that the cells storing the dye stuffs are able to take in antigens, but that their function in the production of antibodies is modified. An extensive review of the subject of the storage of dyestuffs in cells has been published by Jungeblut.<sup>7</sup>

In order to throw light on the question as to whether or not the reticulo-endothelial system has a rôle in the increase of the velocity of the sedimentation of the red blood cells that usually follows inflammation, it was planned to compare the Fahraeus reaction in rabbits that were treated with trypan blue and by injections of pneumococci, with the reaction in rabbits that were treated only by injection of pneumococci.

In passing it may be mentioned that in the first attempts to produce inflammation a sterile solution of aleuronat was used, but the change in the rate of sedimentation of the red blood cells was only moderate. Goodner<sup>8</sup> showed that the injection of pneumococci into the skin of rabbits produces an extensive inflammation that heals in the majority of the animals within ten days.

#### TECHNIC

In all experiments adult, nonpregnant, gray rabbits, weighing from 2 to 2.5 Kg., were used. Usually four subcutaneous injections of 2 cc. of a sterile 1 per cent solution of trypan blue were given on successive days. The skin and sclerae of the animals became intensely blue, and this color failed to increase in intensity on further injections. After these preliminary injections the animals received subcutaneous injections of similar amounts of the dye on alternate days. The persistence of a deep blue color of the blood plasma throughout the experiments was evidence that the rabbits had more dye than they could store. The control animals received no dye.

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5. Gay, F. P., and Clark, A. R.: Reticulo-Endothelial System in Relation to Antibody Formation, *J. A. M. A.* **83**:1296, 1924.

6. Smith, H. P.: *J. Exper. Med.* **51**:395, 1930.

7. Jungeblut, C. W.: *Ergebn. d. Hyg., Bakt., Immunitätsforsch. u. exper. Therap.* **2**:929, 1930.

8. Goodner, K.: *J. Exper. Med.* **48**:1, 1928; **38**:413, 1928.

The sides of the "blocked" and control animals were shaved, and each was given an intracutaneous injection of 0.1 cc. of a 1:100 dilution of an eighteen-hour culture of virulent type I pneumococci. The pneumococci were grown in blood broth and diluted in plain broth.

Before the animals were treated in any way, it was determined that the rates of sedimentation of their red cells were within the accepted normal limits. Readings were made on the day of injection of the pneumococci and on alternate days thereafter until the rates of sedimentation returned to normal. To obtain blood for tests of sedimentation, the ears of the rabbits were shaved and covered with petrolatum, and the marginal vein was cut with a razor. The blood was allowed to flow into a graduated test tube that contained a definite amount of 3.7 per cent solution of sodium citrate. The proportion of blood to citrate was 4:1. The citrated blood, after being shaken, was drawn up to a height of 20 cm. in tubes that had an inside diameter of about 2.5 mm. The lower end of the tube was then forced down into a plaque of modeling clay and carefully adjusted so as to stand vertically. At the end of an hour the height of the column of plasma that separated above the red cells was read in millimeters.

In the majority of the experiments estimations of fibrinogen were made by means of the Abbé refractometer. After centrifugation of the citrated blood, a refractometer reading was taken on the plasma. The plasma was then placed in a water bath at 59 C. for five minutes, which caused a coagulation of the fibrinogen. The samples were then centrifugated at a high speed to throw down the coagulated fibrinogen, after which refractometer readings of the serum were taken. The difference in refraction of the plasma and serum indicated the approximate amount of fibrinogen.

#### EXPERIMENTS

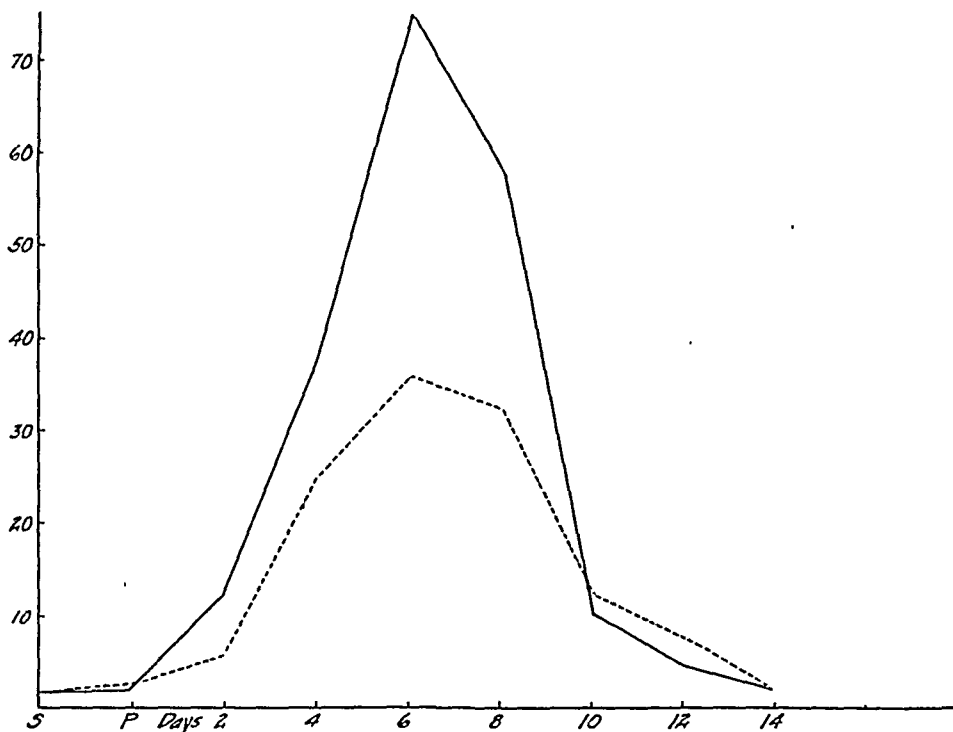
Three individual series of experiments were made. It was found that the injection of trypan blue produced only a negligible increase in the rate of sedimentation. Within forty-eight hours after the injection of the pneumococci all animals showed some increase in the rate of sedimentation, as well as an area of hyperemia about 1 by 1 cm. at the site of injection. The rate of sedimentation of the red blood cells of each animal increased rather rapidly and reached a maximum between the sixth and seventh days after inoculation. During this period the area of hyperemia enlarged to about 6 by 4 cm. These areas became edematous and small areas of necrosis developed in the centers. After this time there occurred a decrease in the rates of sedimentation, equal in rapidity to the rise, and falling to a value on the tenth day equal to that observed on the second day after inoculation. Between the tenth and fourteenth days the rates of sedimentation gradually approached normal. The intensity of the local inflammation increased and decreased as the amount of sedimentation rose and fell; but even after the rates of sedimentation reached normal, there was still visible a slight hyperemia around the site of inoculation in the control animals. Owing to the intense accumulation of dye in the skin of the dye-treated animals, the hyperemia was obscured. In these animals inflammation was evidenced by the presence of heat, edema and necrosis.



## COMMENT

Moderately large variations in the rates of sedimentation occurred in individual animals of the groups that had received dye, as well as in the controls; but when the rates were averaged, the averages showed that the rates of the control animals were almost twice as high as those of the animals that had undergone treatment with dye.

It will be noticed that the sedimentation values of the dye-treated and control groups decreased considerably between the first and the third series of experiments. This is explained by a diminution in the virulence of the stock culture of the pneumococcus, such as usually occurs when a culture is kept on artificial medium for some time.



Composite curves computed from all three experiments. The ordinates show the average height in millimeters of columns of plasma after sedimentation for one hour. The broken line represents the "blocked," the solid line the "unblocked," groups. *S* indicates the day on which "blocking" was started; *P* the day of inoculation with pneumococci.

When averages of the sedimentation rates of all controls and of all animals receiving dye were made and compared (graph), it was found that the rate of sedimentation of red cells in the untreated animals (75 mm.) was more than twice as high as that in the dye-treated animals (31 mm.) at the height of the reaction. It was noted that in individual animals, in averages for each experiment and in averages for all three experiments, the control groups had a lower rate of sedimentation at the beginning of the experiment than the dye-treated groups. A rapid rise in the curve of sedimentation occurred in the dye-treated and control

animals, but the curve of the untreated soon crossed the curve of the dye-treated animals and rose to almost twice the height of the latter. In its fall, the curve of the control animals fell below the curve of the dye-treated, and the two curves finally coincided with normal values. This crossing of the curves, even though it was slight, occurred with such regularity, that it was thought to be indicative of a lag in response to infection in animals that were receiving dye. The lower rates of sedimentation in the dye-treated animals indicated some inhibition of their defense reactions.

During the course of each experiment a few of the dye-treated animals and a few of those receiving no dye died or developed paralysis of the hind legs due to accidents. In such paralyzed animals diarrhea developed, and there was a marked increase in the rate of sedimentation. The observations recorded on such animals to the time of the accidents were allowed to remain in the records and were made use of in the compilation of the curves, but readings subsequent to the accidents were deleted. Animals that showed abnormal rates of sedimentation at the beginning of each experiment, and those in which "snuffles" developed were not included in the records.

By comparing the sedimentation curves of the animals that had received dye and those that had received none, it is seen that the injection of trypan blue ("blocking the reticulo-endothelial system") hinders the rise of the rate of sedimentation of the red blood cells that follows the injection of pneumococci. The hindering effect manifests itself in two ways: 1. The increase in the rate of sedimentation is considerably lower than in the control. 2. In the animals receiving dye the increase in the rate of sedimentation appears somewhat later and persists longer than in the controls.

Since Fahraeus and others showed that an increase in the rate of sedimentation of red cells is due to an increase of fibrinogen, and since these experiments show that injection of trypan blue hinders the increase in the rate of sedimentation accompanying inflammation, it is concluded that the so-called reticulo-endothelial system has a rôle in the increased production of fibrinogen, which was regularly observed in the inoculated rabbits. This experiment does not throw any light on the question as to whether or not the rôle played by the reticulo-endothelial system is a direct or an indirect one. Since these observations show that the injection of trypan blue does not decrease the rate of sedimentation in normal animals, it is probable that the function is indirect, and is active only during the presence of inflammation or other pathologic conditions.

#### CONCLUSIONS

The injection of pneumococci, type I, into the skin of rabbits produces a local inflammation, a rise in the rate of sedimentation of the red blood cells and a concomitant rise in blood fibrinogen.

The rise in the rate of sedimentation parallels in time the development and subsidence of the local inflammation.

The injection of trypan blue does not materially influence the rate of sedimentation of red blood cells in otherwise normal rabbits.

The inflammation at the site of injection of pneumococci is not appreciably influenced by treatment with trypan blue.

The response to infection in dye-treated animals as compared with control animals, as evidenced by changes in the rate of sedimentation of red blood cells, is slower in manifesting itself, is not as intense and persists somewhat longer. It is suggested that the so-called reticulo-endothelial cells play a rôle not only in the production of antibodies, but also in the increase of fibrinogen that accompanies the inflammation caused by the injection of pneumococci. However, the possibility cannot be excluded that the action of the dyestuff is not mediated by these cells but by some other factors.

Dr. Jules Freund, of the Henry Phipps Institute, suggested this problem and made valuable criticisms during the progress of the work.

# General Review

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## PAGET'S DISEASE OF THE NIPPLE

REVIEW OF LITERATURE; CLINICAL AND MICROSCOPIC STUDY OF  
SEVENTEEN BREASTS BY MEANS OF WHOLE SERIAL SECTIONS \*

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Velpeau<sup>1</sup> in 1840 was the first to describe the surface lesion of the disease now known as Paget's disease of the nipple. It remained, however, for Sir James Paget<sup>2</sup> (1874) to discover the intimate association of the epidermic lesion with carcinoma of the breast and to establish the condition that now bears his name as a disease *sui generis*.

Paget's contribution was based on observations of fifteen cases of chronic conditions of the skin of the nipple and areola which he had noted were succeeded by the formation of carcinoma of the breast, usually within one year and at most within two years after their appearance. Paget stated that there was nothing peculiar in the cancers themselves, the majority following an average course with a tendency to local recurrence and metastasis to the lymph glands.

The accuracy of Paget's original description is remarkable. The author records his observations in the following brief, but comprehensive, form:

I believe it has not yet been published that certain chronic affections of the skin of the nipple and areola are very often succeeded by the formation of scirrhous cancer in the mammary gland. I have seen about fifteen cases in which this has happened, and the events were in all of them so similar that one description may suffice.

The patients were all women, various in age from 40 to 60 or more years, having in common nothing remarkable but their disease. In all of them the disease began as an eruption on the nipple and areola. In the majority it had the appearance of a florid, intensely red, raw surface, very finely granular, as if

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\* Submitted for publication, Jan. 30, 1931.

\* The authors gratefully acknowledge their thanks to the Littauer Foundation for having made it possible to conduct the research on which this review is based.

1. Velpeau, A. A. L. M.: *Leçons orales de clinique chirurgicale faites à l'hôpital de la charité*, Paris, 1840-1841, vol. 12.

2. Paget, J. Y.: *On Disease of the Mammary Areola Preceding Cancer of the Mammary Gland*, St. Barth. Hosp. Rep. **10**:87, 1874.

nearly the whole thickness of the epidermis were removed; like the surface of very acute diffuse eczema, or like that of an acute balanitis. From such a surface, on the whole or greater part of the nipple and areola, there was always copious, clear, yellowish, viscid exudation. The sensations were commonly tingling, itching, and burning, but the malady was never attended by disturbance of the general health. I have not seen this form of eruption extend beyond the areola, and only once have seen it pass into a deeper ulceration of the skin after the manner of a rodent ulcer.



Fig. 1.—Sir James Paget

In some of the cases the eruption has presented the characters of an ordinary chronic eczema, with minute vesications, succeeded by soft, moist, yellowish scabs or scales, and constant viscid exudation. In some it has been like psoriasis, dry, with a few white scales slowly desquamating; and spreading far beyond the areola in widening circles, or, with scattered blotches of redness, covering nearly the whole breast.

I am not aware that in any of the cases which I have seen the eruption was different from what may be described as long-persistent eczema, or psoriasis, or by some other name, in treatises on diseases of the skin; and I believe that such cases sometimes occur on the breast, and after months' duration are cured, or

pass by and are not followed by any other disease. But it has happened that in every case which I have been able to watch, cancer of the mammary gland has followed within at the most two years, and usually within one year. The eruption has resisted all the treatment, both local and general, that has been used, and has continued even after the affected part of the skin has been involved in the cancerous disease.

The formation of cancer has not in any case taken place first in the diseased part of the skin. It has always been in the substance of the mammary gland, beneath or not far from the diseased skin, and always with a clear interval of apparently healthy tissue.

In the cancers themselves, I have seen in these cases nothing peculiar. They have been various in form; some acute, some chronic, the majority following an average course, and all tending to the same end; recurring if removed, affecting lymph glands and distant parts, showing nothing which might not be written in the ordinary history of cancer of the breast.

The single noteworthy fact found in all these cases is that which I have stated in the first sentence, and I think it deserves careful study. For the sequence of cancer after the chronic skin disease is so frequent that it may be suspected of being a consequence and must be always feared, and may be sometimes almost certainly foretold.

The accuracy and completeness of Paget's original observations have left nothing unsaid concerning the clinical phases of the disease.

The first histologic studies of the disease were recorded in 1876 by Butlin,<sup>3</sup> who believed that he could demonstrate a direct relationship between the lesion on the surface and that in the breast. He inferred that the eczema was primary and the deeper changes secondary. Butlin's observations were subsequently confirmed by Bowlby<sup>4</sup> (1907).

The most active interest in the subject was aroused in 1899, when Darier<sup>5</sup> announced the discovery that Paget's disease of the nipple was due to "psorosperms." After further studies, however, Darier reached the conclusion that the abnormal cellular elements which he had interpreted as being *Coccidia* were nothing more than epithelial cells that had undergone a special kind of degeneration, to which he applied the term "dyskeratose." The histologic features associated with this state are described by Darier as consisting of epithelial changes in which some of the malpighian cells become isolated and differentiated from the adjacent cells. These cells fail to undergo normal hornification and tend toward individual and special morphologic and chemical alterations. After numerous contradictory reports, it was finally demonstrated that

3. Butlin, H. J.: On the Minute Anatomy of Two Breasts, the Areolae of Which Had Been the Seat of a Long-Standing Eczema, *Med. Chir. Tr.* London 59:107, 1876.

4. Bowlby, A. A.: *Surgical Pathology and Morbid Anatomy*, ed. 5, London, J. & A. Churchill, 1907.

5. Darier, J.: Sur une nouvelle forme de psorospermirose cutanée; la maladie de Paget du mamelon, *Compt. rend. Soc. de biol.* (ser. 9) 1:294, 1889.

the bodies described by Darier and also by Wickham<sup>6</sup> were hydropic, vacuolated cells and not parasites.

Darier's theory that "Paget's cells" are malpighian cells that have become segregated and have undergone dyskeratosis was attacked by Pautrier,<sup>7</sup> who expressed the belief that Paget's cells are truly neoplastic cells with power of invasion. Pautrier concluded that Paget's disease



Fig. 2.—The point of exit of a mammary duct on the surface of the nipple, the probable site of origin of Paget's disease of the nipple. The funnel-shaped opening contains a plug of desiccated epithelial cells and sebum. Note the two sebaceous glands.

of the nipple is not a mere precancerous dyskeratosis, but a genuine epidermotropic cancer, symptomatic of carcinoma of the breast.

6. Wickham, L.: Anatomie pathologique et nature de la Paget du mamelon, *Arch. de méd. expér. d'anat. path.* 2:46, 1890.

7. Pautrier, L. M.: Paget's Disease of the Nipple, *Arch. Dermat. & Syph.* 17:767, 1928.

The precise nature and origin of the peculiar cells to which the term "Paget's cells" is applied has always been and still remains a subject of active controversy and varied opinions. It is to a large extent on the histogenetic interpretation of these cells that a knowledge of the nature of the disease depends.

On the basis of studies of early lesions which seemed to be limited to the epidermis Unna<sup>8</sup> concluded that Paget's cells are peculiarly

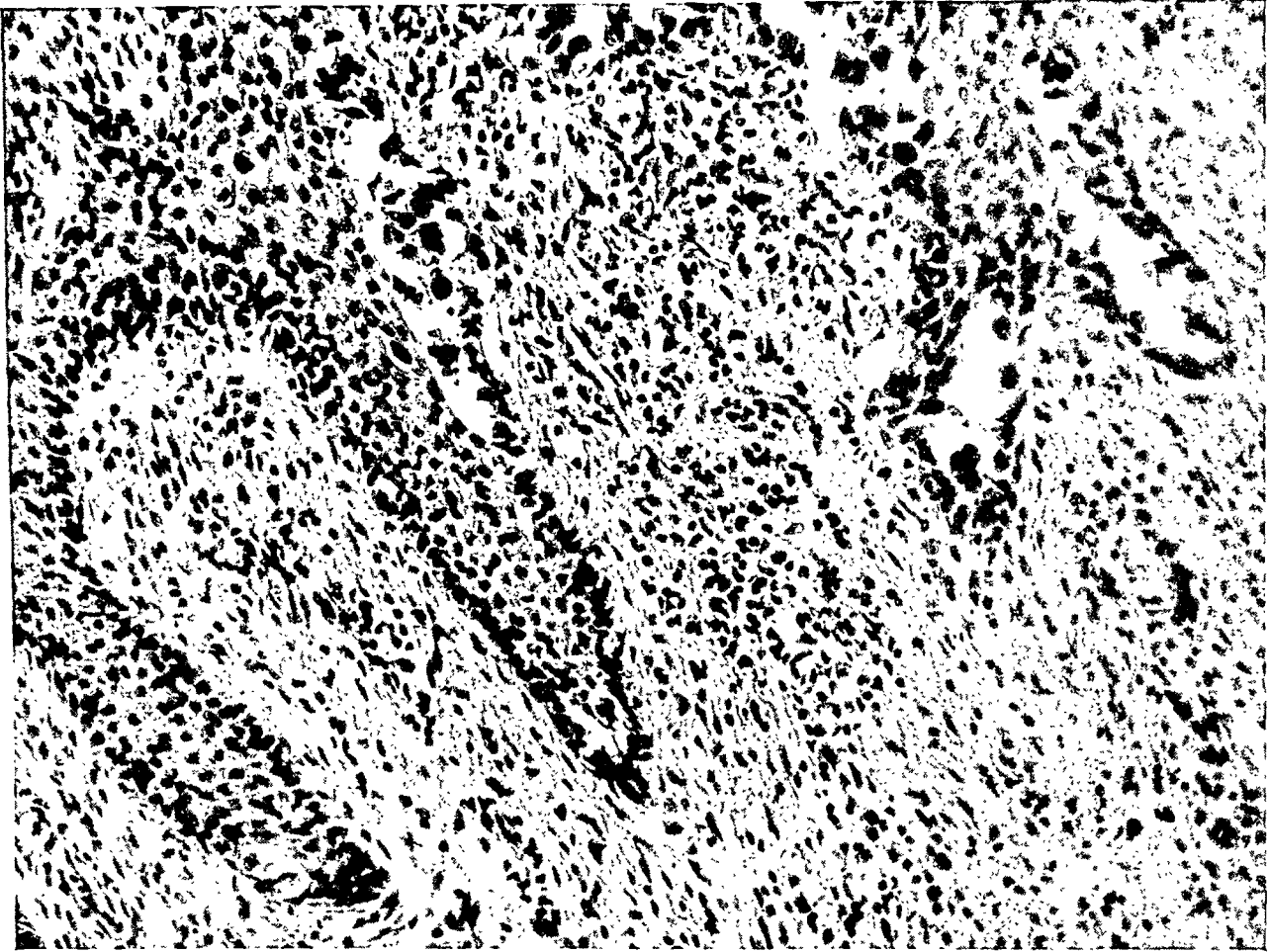


Fig. 3.—Paget's disease of the nipple, apparently beginning in the basal layers of the epidermis. The epithelial cells have become loosened and somewhat disarranged. Paget's cells are visible at several points. These morphologic appearances are not conclusive, and the whole picture may be one of an invasion of the epidermis by malignant cells of an intraduct carcinoma.

edematous epithelial cells arising in the epidermis. Hannemüller and Landois<sup>9</sup> and also Gaarenstroom<sup>10</sup> concurred in this opinion.

8. Unna, P. G.: *Histopathologie der Hautkrankheiten*, Berlin, A. Hirschwald, 1894.

9. Hannemüller, K., and Landois, F.: *Paget's Disease of the Nipple*, *Beitr. z. klin. Chir.* **60**:296, 1908.

10. Gaarenstroom: *Nederl. tijdschr. v. geneesk.* **1**:312, 1913.



Winiwarter<sup>11</sup> saw definite transitional stages between epithelial cells and Paget's cells, and concluded that Paget's cells are of epidermic origin. By special cytologic studies Ludford<sup>12</sup> recently arrived at the same conclusion.

Ewing<sup>13</sup> stated that the long duration of the disease, the wide and superficial extent of the lesion, the occurrence of Paget's disease elsewhere than in the breast and the long absence of any definite tumor render it unlikely that the disease is a peculiar form of invasion of the skin by carcinoma of the ducts and pointed out that in those cases in which the epidermis is invaded by the mammary cancer the invasion rarely takes the form of Paget's disease.

Ewing expressed the belief that Paget's disease occurs in two forms: 1. The characteristic changes in the epithelium affect the epidermis about the nipple, but do not extend deeply into the milk ducts. There is no tumor of the breast. The progress is slow and the prognosis favorable. 2 A rapidly progressive carcinoma of the breast, probably arising from the ducts, extends through the nipple to the skin and spreads widely over the breast. There is usually no definable tumor. The course of the disease is rapid and the prognosis highly unfavorable.

Bloodgood<sup>14</sup> was of the opinion that the disease begins in the epidermis of the nipple, and that the involvement of the ducts in the breast is secondary. Kilgore,<sup>15</sup> in studies of Bloodgood's material, confirms this view.

Arnd<sup>16</sup> stated the belief that Paget's disease of the nipple is a precancerous condition similar to Bowen's "precancerous dermatosis." He was of the opinion that the cells are neither autochthonous carcinomatous cells nor cells that have wandered in, but are overgrown cells that owe their bladder-like formation to their richness in glycogen. These cells he believed can develop multicentrically in the epidermis.

In a recent contribution Drake and Whitfield<sup>17</sup> described a case of Paget's disease of the vulva. Careful histologic study of the lesion showed that the disease began in the basal layer of the epidermis at more than one point. Invasion of the fibrous tissue took place only

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11. Winiwarter, V.: *Arch. f. Dermat. u. Syph.* **85**:239, 1907.

12. Ludford, R. J., cited by Cheatele (footnote 29).

13. Ewing, J.: *Neoplastic Diseases*, ed. 3, Philadelphia, W. B. Saunders Company, 1928.

14. Bloodgood, J. C.: *Paget's Disease of the Female Nipple*, *Arch. Surg.* **8**: 461, 1924.

15. Kilgore, A. B.: *Is Paget's Disease of the Nipple Primary or Secondary to Cancer of the Underlying Breast?* *Arch. Surg.* **3**:324, 1921.

16. Arnd: *Virchows Arch. f. path. Anat.* **261**:700, 1926.

17. Drake, J. A., and Whitfield, A.: *Paget's Disease of the Vulva*, *Brit. J. Dermat.* **41**:177, 1929.

from the epidermis of the surface downward. The authors expressed the belief that "Paget's cells" arise autochthonously in the basal layer and are detached and carried up between the normal prickly cells until eventually they reach the horny layer. Drake and Whitfield objected to the term "precancerous" and pointed out that in Paget's disease there are present three conditions that denote malignancy: namely, invasion, anaplasia of the tumor cells and progressive destruction of surrounding normal tissues. They concluded that as all these conditions are associated in "Paget's disease," even though the progression is so slow as to be almost stationary the disease is an indolent form of carcinoma.

The conception that the lesion on the surface in Paget's disease of the nipple is secondary to carcinoma in the breast was first advocated by Thin<sup>18</sup> in 1881. This publication marks the beginning of a prolonged controversy which to a considerable extent still persists as to the site of origin of Paget's disease of the nipple. This view has been adopted by numerous authorities, including Jacobaeus,<sup>19</sup> Ribbert,<sup>20</sup> Kyrle,<sup>21</sup> Hannemüller and Landois,<sup>9</sup> Muir,<sup>22</sup> Handley,<sup>23</sup> Schambacher,<sup>24</sup> Dietrich,<sup>25</sup> Hirschel,<sup>26</sup> Schmidt,<sup>27</sup> Arzt and Kren<sup>28</sup> and others.

Ribbert<sup>20</sup> agreed with Jacobaeus<sup>19</sup> that the breast is always involved first, the neoplasm reaching the epidermis of the nipple by spreading through the ducts. The adherents to this view point out that histologic pictures resembling Paget's disease are seen when the skin is invaded by a deep-seated carcinoma from below. Thus Borst described the histologic features of Paget's disease in the epidermis surrounding a carcinoma of the lip. The opponents of this view point out that histologic pictures resembling Paget's disease as a rule do not occur when a deep-seated mammary carcinoma affects the nipple (Ewing,<sup>13</sup> Cheatle,<sup>29</sup> Deaver and McFarland<sup>30</sup>).

18. Thin, G.: On the Connection Between Diseases of the Nipple and Areola and Tumors of the Breast, Tr. Path. Soc., London, **32**:218, 1881.

19. Jacobaeus, H. C.: Virchows Arch. f. path. Anat. **178**:124, 1904.

20. Ribbert, H.: Ueber der Pagetkrebs, Deutsche med. Wchnschr. **31**:1218, 1905; Beiträge zur Entstehung der Geschwülste, Bonn, F. Cohen, 1906.

21. Kyrle, J.: Drusenkrebs der Mamma unter den klinischen Bilde von Paget's Disease, Arch. f. Dermat. u. Syph. **83**:187, 1907.

22. Muir, R.: Paget's Disease of the Nipple and Its Relationships, J. Path. & Bact. **30**:451, 1927.

23. Handley, W. S.: On Paget's Disease of the Nipple, Brit. J. Surg. **7**:183, 1919-1920.

24. Schambacher, A.: Deutsche Ztschr. f. Chir. **80**:332, 1905.

25. Dietrich: Verhandl. d. deutsch. Path. Gesellsch. **17**:329, 1914.

26. Hirschel: Beitr. z. path. Anat. u. z. allg. Path. (supp.) **7**:573, 1905.

27. Schmidt, M. B.: München. med. Wchnschr. **59**:22, 1912.

28. Arzt and Kren: Arch. f. Dermat. u. Syph. **148**:254, 1925.

29. Cheatle, G. L.: Paget's Disease of the Nipple, Brit. J. Surg. **11**:295, 1923.

30. Deaver and McFarland: The Breast: Its Anomalies, Its Diseases and Their Treatment, Philadelphia, P. Blakiston's Son & Company, 1917.

Handley<sup>23</sup> believed that carcinoma precedes and causes Paget's disease on the surface. Thus Handley considered that carcinoma begins in the smaller ducts of the breast and usually, without producing a palpable tumor, widely permeates the lymphatic vessels of the nipple. According to this view, the changes that occur on the surface of the nipple are due to edematous swelling of the papillae of the dermis resulting from lymphatic obstruction.

The view that the disease is merely an extensive intra-epidermal invasion of the nipple from a primary carcinoma in the underlying breast has been specially advocated by Jacobaeus,<sup>19</sup> Ribbert,<sup>20</sup> Schambacher,<sup>24</sup> Masson<sup>31</sup> and more recently Muir.<sup>22</sup>

Masson<sup>31</sup> raised the question as to the nature of "Paget's cells" and expressed the belief that Paget's disease is an epithelioma that has originated in the epithelium of the openings of the ducts. For a long period the disease affects only the epidermis, subsequently invading the connective tissue. During the whole period of this invasion of the epidermis it is neither a dyskeratosis nor a precancerous lesion, but a carcinoma. By careful examination of the lesions in Paget's disease he has been able to discover, besides the spherical cells, other cells with a more or less lobed cytoplasm comparable to that of leukocytes in motion.

The belief that malignant epithelial cells are endowed with the property of ameboid-like motion has been expressed by various investigators, particularly by Muir. Muir<sup>22</sup> believed that both Paget's disease of the nipple and the outbreak of carcinoma in the upper ducts of the breast are parts of the same process. He considered that "Paget's cells" do not originate in the epidermis of the nipple, but that the lesion in the epidermis represents a direct intra-epithelial invasion by malignant cells from carcinoma in the upper ducts of the nipple. He noted a marked similarity in appearance between the altered cells in the epidermis and those in the deeper ducts. The advance of the epithelium of the ducts occurs, according to Muir, by direct intra-epithelial invasion, as well as by an "undermining process." Microscopic appearances are depicted, which are interpreted as demonstrating this method of spread. Muir also believed that a squamous epithelioma arising in the epidermis may also spread in and among the epithelium of the epidermis. Dunn<sup>32</sup> reported a case of carcinoma of the anus containing mucigenous cells, in which this peculiar migration of carcinomatous cells apparently had occurred. Fraser<sup>33</sup> (1928) reported microscopic studies of two specimens of Paget's disease of the nipple, the results of which seem to support Muir's theory.

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31. Masson, P.: *Bull. Soc. franç. de dermat. et syph.* **32**:6, 1925.

32. Dunn, Shaw, cited by Cheatle (footnote 29).

33. Fraser, J. F.: *Bowen's Disease and Paget's Disease of the Nipple: Their Relation to Dyskeratosis*, *Arch. Dermat. & Syph.* **18**:809, 1928.

In 1923, one of us (Dr. Cutler<sup>29</sup>) described eight cases of Paget's disease of the nipple in which the breast was examined by cutting serial sections of the whole gland. A ninth case was reported the following year (1924). Based on these studies this one of us felt justified in stating the following conclusions: 1. Paget's disease of the nipple is carcinoma. 2. Carcinoma in the breast, with which Paget's disease of the nipple is usually associated, is a primary carcinoma of the epithelium of the breast. 3. The connection between the two lesions is that the agent which is inducing Paget's disease on the surface is also concerned in inducing primary carcinoma in the epithelial cells of the underlying breast, which is reached by means of the mammary ducts.

Cases of Paget's disease in situations other than the nipple and areola yield important information as to the nature of the disease, particularly as regards its histogenesis. Thus the involvement of extramammary structures by Paget's disease is regarded by some as formidable evidence against the theory that Paget's disease on the surface of the nipple is secondary to carcinoma in the deeper structures of the mammary gland.

In 1910, Hartzell<sup>34</sup> reported a case of Paget's disease of the forearm in which a diagnosis of eczema had been made. Histologic examination showed typical Paget's disease in the periphery of the lesion and melanoma in the central ulcerated portion. The lesion had begun in a pigmented mole which had been traumatized. Hartzell collected from the literature reports of eighteen cases of extramammary Paget's disease. The diagnosis in some of these examples is highly doubtful. Further compilations from the literature were made by Deaver and McFarland<sup>30</sup> in 1918 and by Pautrier<sup>7</sup> in 1928. The following résumé has been taken partly from these sources.

Crocker<sup>35</sup> is credited with having recorded the first observation of extramammary Paget's disease in 1887. The glans penis and scrotum were affected in a man, aged 61. There is some question as to whether this lesion may not have been a carcinoma having its origin in a sweat gland.

Darier and Couilland<sup>36</sup> (1893) reported a case of Paget's disease of the anus and perineum. In Tommasoli's<sup>37</sup> case the disease affected the penis of a man, aged 56. Here the histologic diagnosis was not absolutely positive. In 1894, Ravogli<sup>38</sup> reported a case of Paget's dis-

34. Hartzell, M. B.: *J. Cutan. Dis.* **28**:8, 1910, cited, *Arch. f. Dermat. u. Syph.* **104**(52):355, 1910.

35. Crocker, quoted by Fox and MacLeod: *Tr. Path. Soc. London* **40**:187, 1889.

36. Darier and Couilland: *Bull. Soc. franç. dermat. et syph.* **4**:25, 1893.

37. Tommasoli: *Gior. ital. d. mal. ven.* **28**:542, 1893.

38. Ravogli: *J. Cutan. Dis.* **12**:222, 1894.

ease affecting the nose of a man, aged 67. The lesion was of six years' duration. Winfield<sup>39</sup> (1896) reported a case in which the disease affected the mucocutaneous surface of the lower lip and had lasted four years. The true nature of the disease in both these cases is in doubt. The case of Rolleston and Hunt<sup>40</sup> in which the pubes was the seat of a lesion of eight years' duration is not clearly one of Paget's disease. Dubreuilh's<sup>41</sup> patient was a woman, aged 51; the lesion affected the vulva and was present for three years. In the case recorded by Fordyce<sup>42</sup> the lesion affected the buttock of a woman, aged 60, and had lasted six years. Holzkecht<sup>43</sup> reported a case in which the axilla had been the seat of Paget's disease for twelve years. Fox and MacLeod<sup>44</sup> reported Paget's disease of the umbilical region of eleven years' duration in a man, aged 65. Rosenberg's<sup>45</sup> patient was aged 70; the lesion affected the vulva. Milligan's<sup>46</sup> patient, a woman, aged 31, suffered from Paget's disease of the umbilical region and was cured by radium. In Sequeira's<sup>47</sup> patient, a man, aged 82, Paget's disease affecting the glans penis, and carcinoma of the bulbus urethra was found at autopsy. Towle<sup>48</sup> reported a case of Paget's disease of twenty-five years' duration affecting the interscapular region.

Satani's<sup>49</sup> case is of considerable interest. The lesion occurred in the axilla of a man, aged 74, and was of six years' duration. The sebaceous glands had been invaded. The central part of the lesion was an epithelioma. The author was of the opinion that Paget's cells may originate in epidermic cells or cells of the sebaceous glands. Drake and Whitfield,<sup>17</sup> after studying a case of Paget's disease of the vulva, stated that the disease probably originates in the basal layer of the epidermis.

#### CONSIDERATION OF SEVENTEEN BREASTS AFFECTED BY PAGET'S DISEASE OF THE NIPPLE

We have studied seventeen cases of Paget's disease of the nipple in which radical mastectomy had been performed. Serial sections of the whole nipple and breast were subjected to microscopic examination.

39. Winfield: Brooklyn M. Soc., 1896.

40. Rolleston and Hunt: Tr. Path. Soc., London **48**:211, 1897.

41. Dubreuilh: Brit. J. Dermat. **13**:407, 1901.

42. Fordyce: J. Cutan. Dis. **21**:567, 1903.

43. Holzkecht: Wien. klin. Wchnschr. **16**:1318, 1903.

44. Fox and MacLeod: Brit. J. Dermat. **16**:41, 1904.

45. Rosenberg: Monatsch. f. prakt. Dermat. **49**:235, 1909.

46. Milligan: Brit. J. Dermat. **23**:411, 1911.

47. Sequeira: Tr. Roy. Soc. Med. **33**:5, 1912.

48. Towle: J. Cutan. Dis. **24**:27, 1912.

49. Satani: Brit. J. Dermat. **32**:117, 1920.

The practical part of the investigation of these breasts was rendered simple and the topographic changes in them pictorially perfect by the method of cutting microscopic serial sections of the whole breast in each case. For purposes of gaining knowledge and demonstrating gross and minute changes in the breast no other process is so simple, exact and clear. A method fulfilling these requirements is more essential in the study of Paget's disease than in the study of any other in view of the fact that so much confusion exists in the minds of pathologists as to the precise conditions in the underlying breast. Small and apparently insignificant areas are actually often the seat of important pathologic changes, which can be easily missed by the method of cutting small sections and which are easily demonstrated by the method of cutting whole sections. Both means of investigation are laborious, but the method of whole sections is the more simple of the two.

#### THE CLINICAL SIGNS EVOKED BY THE PATHOLOGIC PROCESS

Paget's disease of the nipple affects other parts of the body as well as the structure from which it derives its title. Besides the nipple, the prepuce, the skin and the vulva have been described as its sites of origin. The disease is rare at any part. Perhaps the nipple is the commonest situation. It is more common in women than in men.

The clinical signs associated with the disease are distinct and classic. The precise nature of the changes that cause the clinical signs is the subject of controversy. Different observers describe different changes as being causal. If all these observers are correct, it must be assumed that different changes can cause the same clinical signs.

The present time does not appear to be ripe for a dogmatic statement on the subject. At present the solution of the matter depends on morphologic appearances to which different interpretations can be justly applied. Some conclusive help may be gained in this direction when all the cells taking part in the process or processes have been subjected to the scrutiny of expert cytologists. The disease is so rare that we have been able to subject only one case to an efficient cytologic examination since we have come to this conclusion. The results of this cytologic examination, conducted by Dr. R. J. Ludford,<sup>12</sup> are so important in many ways that it may greatly assist the solution of the problem so far as morphology can be valuable. Without finally being able to solve the matter, we shall attempt to reach as near the goal as we consider possible. In the historical description of the disease the various authorities to whom reference will be made have been mentioned, as well as the conclusions that they have respectively reached. As the only features concerning which most authorities agree are the clinical signs, it would be well to describe these first.

The clinical signs are as follows: Only one nipple is affected, and the change always begins on the surface at the top of the nipple. There is a dry, bright-red, raw-looking surface, covered here and there by small white scales. The area of disease spreads slowly, regularly and

centrifugally in all directions and may eventually occupy an extensive surface of the body. At the margins of the lesion, fresh, isolated, minute foci of the disease appear to arise spontaneously and subsequently spread to join and enlarge the affected parts. The edges are firm and are defined on palpation. The scales are chiefly composed of shed degenerated epithelial cells. The cells are large and vacuolated; their nuclei may be crescentic and marginal or round and central. They have been termed "Paget's cells" and in former days were regarded as psorosperms. Lymphatic glands in the axilla of the side first affected may be enlarged. Other groups of lymphatic glands may also be affected as the disease reaches fresh areas of skin. These are the clinical signs that have evoked so many theories as to their cause.

The probability is that the clinical signs of Paget's disease of the nipple are most typical when the disease begins in and spreads from the epidermis of the opening of a duct or in the epidermis of the surface of the nipple.

The following pathologic conditions have been included as causing the clinical signs that are characteristic of Paget's disease of the nipple: (1) carcinoma of the epidermis of the surface of the nipple; (2) carcinoma arising in the squamous epithelium in the opening of a duct; (3) carcinoma arising in the columnar epithelium in the region of the upper part of a duct, and (4) carcinoma arising in the depths of the breast, metastatic deposits from which have reached the epidermis of the nipple by spreading upward from the subepidermic connective tissue. In early and sometimes in late conditions the seat of origin in conditions 1, 2 and 3 is clearly distinguishable.

The difficulty of determining by morphologic examination alone which of these conditions is present in any particular instance is manifested on consideration of the following possibilities: 1. Squamous cells when they become carcinomatous may at once lose all morphologic evidence of their origin. 2. It is impossible to say whether glandular epithelium of the breast does or does not lose morphologic indications of its origin when the cells reach and spread in the epidermis. It is impossible to say that carcinomatous cells have not arisen in glandular epithelium of the breast merely because there is no cytologic evidence of their being secretory. A carcinomatous cell arising from mammary epithelium may lose its biologic secretory function when it reaches and spreads among epidermic cells.

#### SITE OF ORIGIN AND NATURE OF THE PATHOLOGIC PROCESS

In all of our seventeen examples the disease began in the surface at the top of the nipple. There is only one set of structures in the top of the nipple and nowhere else in its construction: the exits of the mam-

mary ducts. The probability is that the disease begins in these openings of ducts. The openings of the ducts are usually plugged by desiccated shed epithelial cells and sebum. The constant presence of the plugs may act as an irritant and also as an aid to the entrance of other irritants. The lowest layer of epidermis contains pigmented cells, the presence of which may serve the purpose of some authors in correlating the epithelial neoplasia that occurs in Paget's disease with melanoma. All observers are also agreed on the following points:

1. The pathologic changes exhibit their least advanced stage at the extreme margins of the rash, and occur in the lower and basal layers of the epidermis.

2. So-called "Paget's cells" are present throughout the lesions. They are present in greater numbers and are more typical in the least advanced stage of the disease, viz., at the margin, than they are in the older parts of it.

3. In the older parts of the lesion there commonly occurs an epithelial neoplasia that looks malignant, although it is confined within the normal boundaries of the epidermis.

4. The nipple disappears as a prominence on the surface of the lesion. Its disappearance is due, in most instances, to its retraction. Its retraction is due to the contraction of cicatricial tissue that forms in subepidermal connective tissue at its surface and around the openings of the ducts.

5. Accompanying the rash on the surface there are changes in the subepidermal connective tissue. They occur early and simultaneously with the least advanced changes at the margins of the rash. The situation of their earliest manifestations is between the elastica and the epidermis. By means of these changes, either the epidermis is raised or the elastica is depressed downward. In this situation the scene is one of activity. Plasma cells accumulate in great numbers. Lymphocytes and macrophages also collect, and recently formed connective tissue increases in number with the age of the lesion. The elastica undergoes diffuse and scattered hyperplasia, which is more marked in some instances than in others. It can always be traced in continuity with the normal elastica beneath the epidermis and around the mammary ducts. In later stages the fibers of the elastica entangle the plasma cells, lymphocytes and macrophages. Polymorphonuclear leukocytes are also present. The most marked feature, however, is the great numbers of the plasma cells.

The controversy rages between two chief theories: The first is that the disease is purely epidermic in origin. This involves subsidiary theories: (a) that it is a degenerative process, and (b) that it is a proc-





Fig. 4.—Highly atypical and malignant-looking epithelial neoplasia filling and distending the upper part of a duct near the surface in Paget's disease of the nipple. The process is still confined within the walls of the duct (intraduct carcinoma).

ess of epithelial neoplasia. The second theory is that all the component parts of Paget's disease are malignant cells of a carcinoma of the ducts, which have spread among the normal epidermic cells. A discussion on these two points would be long, involved, tedious and inconclusive, for the chief reason that morphologic appearances are not decisive enough to be generally convincing.

We believe that our seventeen cases demonstrate that:

1. The clinical signs of Paget's disease of the nipple can be caused by changes in the epidermic cells alone. "Paget's cells" can be traced in their development from epidermic cells through all stages of their formation.

2. We cannot convince ourselves that the formation of "Paget's cells" and the early separation of basal epidermal cells from each other are preliminary to malignant disease of these cells, although we have no evidence to the contrary.

It is difficult, by morphologic examination alone, to be sure of the origin of a cell after it has become malignant; and it is a question whether a mild form of malignancy of an epidermic condition can be established—as Whitfield and Drake believe it can be—by the frequent occurrence of mitosis in epidermic cells, in a constantly and widely progressing epidermic disease. All that can be said of the matter is that these authors may be right, and that no one can contradict them in the present state of morphologic knowledge.

3. Our seventeen cases confirm Professor Muir's statement that in the majority of instances the clinical signs of Paget's disease of the nipple are associated with an invasion among epidermic cells by malignant cells that have spread from a carcinoma in the columnar cells in the extremity of a duct. These cases show that associated with this invasion there are also, in the majority of instances, the characteristic pathologic changes in the epidermic cells.

We believe it is probable that the classic clinical signs of the disease cannot be evoked in these examples of invasion unless they are associated with the purely epidermic changes also.

When the two processes coexist, it is impossible to state which was the first to appear.

Special cytologic studies of a case of Paget's disease of the nipple conducted by R. J. Ludford<sup>12</sup> have yielded information of paramount importance as to the histogenesis of the lesion. Ludford was able to demonstrate that the sessile convex lumps in the mammary region were composed of mammary carcinomatous cells. He also showed that in the wide marginal zone that exhibited the classic signs of Paget's disease there was no evidence of epithelial neoplasia. In this zone he discerned epidermic cells in all stages of alteration into "Paget's cells."

The process is not neoplastic, but degenerative. The conclusion is that degeneration of epidermic cells can cause all the clinical signs of Paget's disease.

The degenerative process in the epidermis has been attributed by some authors to lymphatic obstruction caused by the plugging of sub-jacent lymphatic vessels by carcinomatous cells. To this view we are fundamentally opposed, since we have in our possession many examples in which these lymphatic vessels are plugged by carcinomatous cells with no Paget's disease of the nipple. We have also other examples in which the whole of the basal areas of the nipple itself are occupied by dense masses of carcinoma, while the epidermis of the nipple is normal. There are examples of Paget's disease in other areas of the body where there is no plugging of lymphatic vessels by carcinomatous cells.

We draw attention here to a fact that seems to be invariably neglected in descriptions of Paget's disease of the nipple. The fact is lost sight of in the confusion and controversy arising from the attempt to explain the causes of the clinical signs of Paget's disease. Yet from an etiologic point of view the fact is important and significant. The fact is this, and Muir<sup>22</sup> agrees that this is a fact: In the depths of the breast three kinds of epithelial neoplasia often occur totally unconnected with spread of disease or transplantation of disease from elsewhere: (1) papillomas in the ducts, (2) malignant looking epithelial neoplasia in terminal ducts and acini and (3) carcinoma arising from a primary tumor in the terminal ducts and acini. It is from this carcinoma that the secondary deposits are usually derived when the lymphatic glands contain carcinomatous cells in Paget's disease of the nipple. The observation logically suggests that the agent inducing the epithelial neoplasia in the depths of the breast reached that situation either by passing down the ducts or by being borne there by the blood from the disease on the surface.

The first part of the problem to be dealt with is the question whether or not the changes in the breast are connected with the changes in the lesion at the surface. It may be said at once that morphologic appearances alone prove that there is a direct connection between the two. The connection has a distinct bearing on the etiology of carcinoma in the breast, in particular, and on the problem of carcinoma, in general.

Whatever is the true explanation of "Paget's cells" and of the malignant epithelial neoplasia with which they are definitely correlated there can be no doubt that these two processes may be limited to the epidermis of the nipple and of the openings of the ducts on its surface and to the upper ducts. From our point of view, the most remarkable fact about the disease is that deeper down in the breast, after long intervals during which the ducts are normal, papillomas may form in

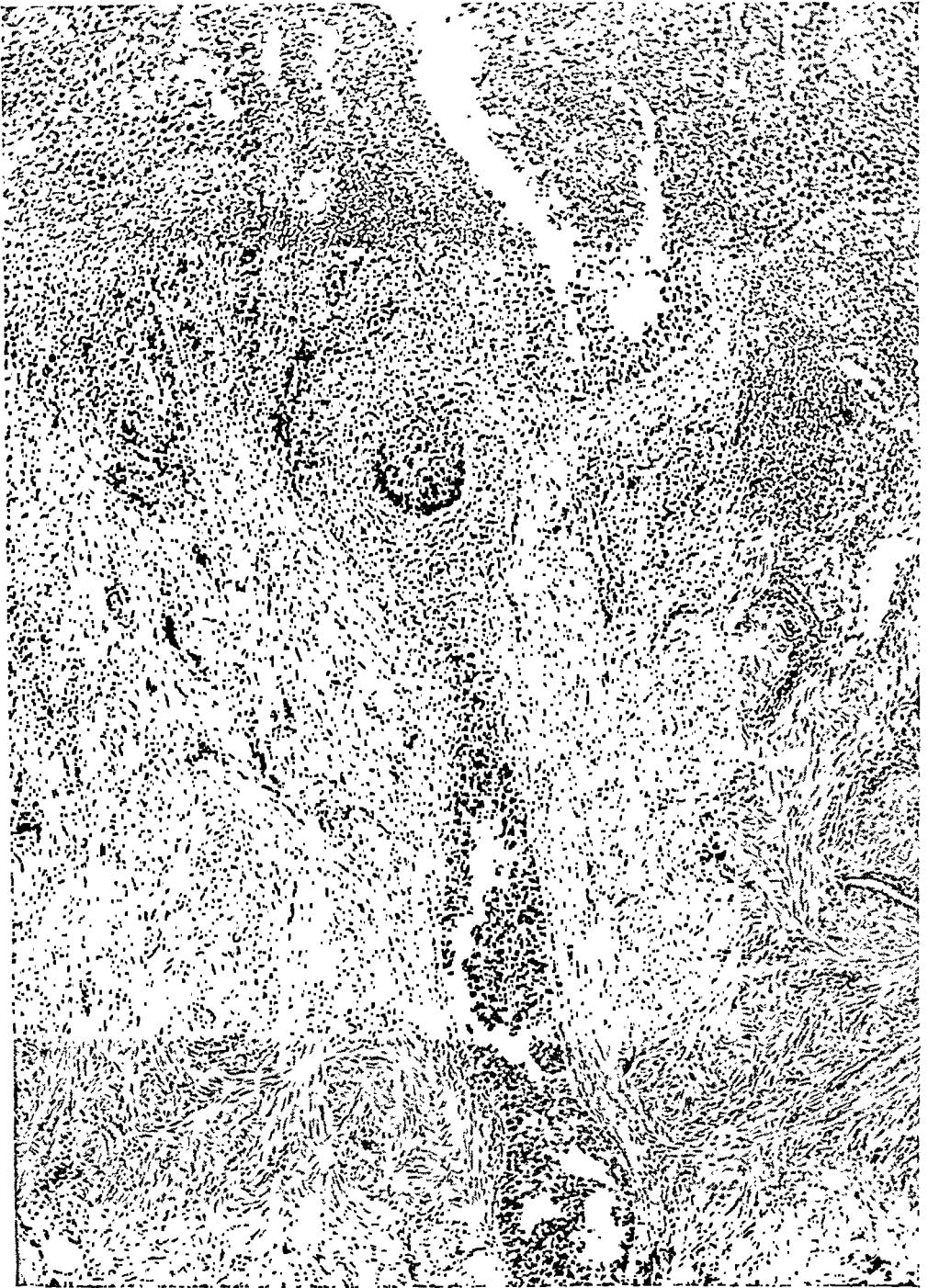


Fig. 5.—Paget's disease on the surface of the nipple, and carcinoma affecting the upper part of a duct, which is filled with an atypical epithelial neoplasia of highly malignant appearance.

the ducts, and that in the terminal ducts and acini epithelial neoplasia forms spontaneously in the epithelium that lines these structures. The neoplasia may look benign, it may look malignant and be confined within normal boundaries and it may be in a state of carcinoma. When these changes appear spontaneously in the peripheral parts of the gland, they almost invariably lead to the same upper parts of the ducts in which

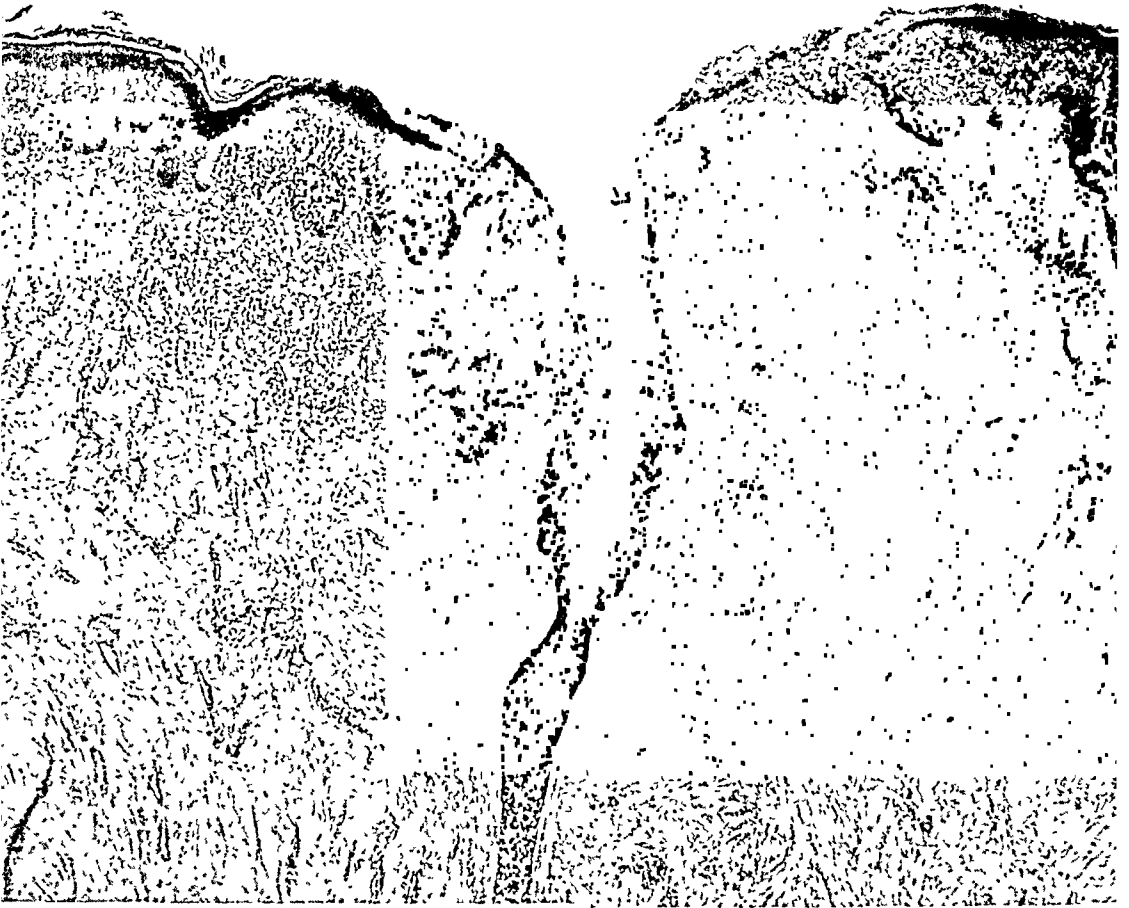


Fig. 6.—Paget's disease on the surface of the nipple; upper part of a duct and its opening on the surface filled with malignant-looking epithelial neoplasia. Note the anatomic continuity between the disease on the surface and that in the duct, also the intra-epidermal spread of malignant cells into the squamous epithelium of the opening of the duct.

Paget's disease exists. In all the seventeen cases, except one, epithelial neoplasia exists in the terminal ducts and acini which lead into, first, normal ducts and finally into the Paget's disease on the surface. While emphasizing this relationship between the disease in the upper regions and that in the underlying regions of the breast, we do not intend to



Fig. 7.—Epithelial neoplasia of malignant appearance in three ducts and the acini of one of them after a long interval during which the ducts were normal. The upper parts of these ducts were also the seats of a similar epithelial neoplasia which was continuous with typical Paget's disease on the surface of the nipple. The problems here are: 1. Has carcinoma arisen multicentrically in these ducts? 2. Did the carcinoma begin in one duct and spread intra-epidermally in the nipple till it reached the openings of these three ducts down which it continued to spread intra-epithelially?



Fig. 8.—Paget's disease of the nipple showing typical Paget's cells in the basal layer of the epidermis; upper parts of mammary ducts distended by highly atypical epithelial neoplasia of malignant appearance (intraduct carcinoma). After long intervals during which the ducts were normal, the terminal ducts and acini showed similar epithelial changes.

infer that the epithelial neoplasias in both situations are in the same biologic state of activity. The facts prove the contrary.

The epithelial neoplasia in the upper regions remains for a considerable time confined within its normal boundaries, although it may be spreading beneath and among normal epithelial layers in these regions. Even when transgression of normal boundaries occurs, the invading cells remain localized and do not usually affect the axillary lymphatic glands. In other words, the disease in these upper regions usually remains locally malignant. When epithelial cells escape from the epithelial neoplasia in the terminal ducts and acini, they rapidly affect the axillary lymphatic glands. When carcinoma in Paget's disease involves lymphatic glands, the involvement is usually secondary to carcinoma in the terminal ducts and acini and not to the surface disease. Biologically the epithelial neoplasia that arises spontaneously in the lower regions is in a more highly malignant state of activity than that occurring in the upper regions of the same ducts.

A subjacent carcinoma may induce necrosis of epidermis without infiltrating it and cause an ulcer. In these instances there is no appearance of the classic signs of Paget's disease. We have never seen an example of Paget's disease accompanied by papillomatous carcinoma in a duct at the opening or immediately below the opening. The papillomatous tumors arising in these situations ulcerate the surface of the nipple, which exhibits no signs of Paget's disease. The surfaces of these ulcers are not flat or scaly, and with a hand lens they are often seen to be composed of small sessile elevations.

A subjacent carcinoma may infiltrate the epidermis of the nipple from below and finally lead to shedding of horny layers and give rise to another type of ulcer. This lesion may or may not cause the classic clinical signs of Paget's disease. Microscopically it differs from Paget's disease by the absence of epithelial neoplasia in the openings of the ducts.

Why carcinoma infiltrating the epidermis of the nipple from below should give rise to clinical signs of Paget's disease is probably explained by stating that the effect produce in the epidermis of the nipple and areola is different from that in the skin when it is infiltrated from below by a subjacent carcinoma. There are no signs of Paget's disease in the skin that has been infiltrated by carcinoma from below. "Cancer en cuirasse" for example exhibits no signs of Paget's disease.

Another inquiry is pertinent at this point. Can any other type of carcinoma arising on the surface at the top of the nipple induce epithelial neoplasia in the terminal ducts and acini? Unfortunately, other types of carcinoma arising on the surface of the nipple are exceedingly rare. We have one specimen of carcinoma that occurred on the surface at the top of the nipple which began multicentrically in the sebaceous



glands. In the terminal ducts and acini of the breast underlying this disease there is epithelial neoplasia of malignant appearance, although it is confined within normal boundaries. There is no spread of carcinoma into the upper regions of the ducts, although they opened onto the diseased surface. In this instance the etiologic question arises: Did the factor that induced carcinoma of the sebaceous glands on the surface also induce the malignant looking epithelial neoplasia in the terminal ducts and acini of the breasts?

*Epithelial Changes.*—The general impression gained by the inspection of these cases supports the conception that carcinoma arising in the upper ducts proper and in the openings of ducts is associated with those changes that cause the clinical signs of Paget's disease of the nipple. These clinical signs may accompany a carcinoma that has spread into the epidermis by direct growth, or they may be caused by a carcinoma of multicentric origin, arising spontaneously in the epithelium of a duct, the epithelium of the opening of the duct and also the epithelium in the epidermis of the nipple. Morphologic examination could explain all these observations equally well, and cannot assist in solving this problem.

In this connection attention should be drawn to two types of examples: 1. In one of the seventeen specimens there is no neoplasia in the superficial lesion; all that is seen is the formation of "Paget's cells" from the basal layer of epithelium in the epidermis of the nipple. 2. In a second specimen the same observations are to be made in the wide marginal zone of a lesion that is very extensive. These examples prove that the clinical signs of Paget's disease of the nipple can be induced by the change of epidermic epithelium into "Paget's cells." The almost invariable association of these pathologic appearances with epithelial neoplasia renders the combination almost specific in inducing the classic signs of the superficial disease of Paget in its relation to the nipple. Morphologic appearances alone cannot always be decisive in solving whether the epithelial neoplasia is merely a spread into the epidermis from carcinoma elsewhere or whether carcinoma is beginning multicentrically in the epithelial cells among which it is present. Examples of Paget's disease of the nipple occurring in the skin in other parts of the body do not materially assist in the solution of this matter.

*Changes in Connective Tissue.*—Changes in the connective tissue in Paget's disease chiefly concern (1) hyperplasia of the subepithelial connective tissue, (2) hyperplasia elastica and (3) accumulations of lymphocytes in the pericanalicular and periacinous tissues in different degrees of intensity.

Hyperplasia of the subepithelial connective tissue throughout these breasts affects the ducts toward and including their acinous termina-

tions, which contain epithelial neoplasia. It may also be seen affecting the apparently normal parts of the breast in a regular, diffuse manner and without epithelial change. In some branches of the involved ducts hyperplasia of the subepithelial tissue is so marked and undergoes such extensive hyaline degeneration that no epithelial cells can be seen in them.

When hyperplasia elastica occurs, it is usually present over the whole breast; but in those ducts and acini that are affected by the neoplastic epithelial hyperplasia it is so enormous as to render these particular parts discernible on macroscopic examination of the stained section of the whole breast.

The accumulations of lymphocytes appear to follow no definite rule. They occur in the pericanalicular and periacinous connective tissue of ducts that contain neoplastic epithelial hyperplasia and around those in which the epithelium appears to be normal. They can also be seen in parts where carcinoma is rampant. In some instances lymphocytes can be seen spreading from these collections into the walls of a duct. The presence of these lymphocytes is secondary to the lesions occurring in the breast and cannot be taken to indicate factors predisposing to those lesions.

#### CLINICAL CONSIDERATIONS

*Symptoms.*—The disease is unilateral and begins on the surface at the top of the nipple as a scaly, infiltrated, bright-red patch. The nipple is sore, very often itches and sometimes is affected by a burning sensation. It is sometimes moist and at other times dry. The base and margins of the lesion are hard, abrupt and well defined. Its irregular shape is due to the fact that occasionally some parts of it affect the skin more rapidly than others. The surface does not bleed, unless it is injured by rubbing or removal of adherent dressings. The spread is slow, and although the surface disease usually remains limited to the nipple and areola, it may extend far beyond these boundaries in all directions. As the disease progresses, the nipple becomes flattened and finally disappears either from atrophy or retraction. The lymphatic glands are not usually enlarged, and when they are, they do not usually contain metastatic cells, even though the Paget's disease is extensive and of long duration. When the lymphatic glands contain metastatic growth, it is an undoubted sign that carcinoma exists in the underlying breast. In other words the epithelial neoplasia in Paget's disease of the nipple is a slow locally infiltrating process and does not as a rule give rise to metastatic deposits. As indicated in foregoing paragraphs carcinoma deep in the underlying breast is a common complication of Paget's disease on the surface. When this complication is advanced, the condition of the underlying breast exhibits the signs usually associated with carcinoma of this gland. The carcinoma may exist without

revealing clinical signs. In fact, the disease in the subjacent breast may be so masked that its discovery is attainable only by careful microscopic examination of serial sections of the whole breast. (Eller and Anderson<sup>50</sup> found intraduct carcinoma in three cases of Paget's disease of the nipple.)

The slow development and progress of the epidermic lesion is characteristic of this disease. Examples of unusually long duration are recorded by various observers. Kaufmann<sup>51</sup> noted a case in a woman, aged 85, in which the ulceration had been in progress for twenty years and covered the entire surface of the thorax. A similar case was described by Vignolo-Lutate.<sup>52</sup> Jamieson<sup>53</sup> described a case in which Paget's disease existed on the surface for twenty years without the occurrence of carcinoma in the underlying breast. Masland and Babcock<sup>54</sup> (1899) reported a case in which the disease completely destroyed the nipple without the occurrence of carcinoma.

One patient in the series on which our study is based was 72 years of age. The lesion began seven years previous to examination and covered a wide area of the anterior, lateral and posterior walls of one side of the chest. On microscopic examination the central portion of the lesion showed carcinoma.

*Diagnosis.*—There are five chief conditions of the nipple and areola that must be differentiated from Paget's disease of the nipple, namely: eczema; carcinoma that has invaded the nipple and areola from its origin in the subjacent breast; carcinoma arising in the sebaceous glands on the surface at the top of the nipple; syphilis, and diffuse, chronic, subepithelial, possibly infective lesions of the nipple.

Eczema is usually bilateral and more frequently occurs in younger people. Paget's disease is always unilateral and occurs most commonly in persons between 40 and 60 years of age. The nipple in eczema retains its shape and prominence. In Paget's disease it is flattened or retracted and gradually disappears as a structure of the surface. Eczema is intermittent and amenable to treatment; Paget's disease is constant and slowly and continually progressive. The eczematous surface is soft and pliable and the margin of the lesion exhibits no induration on palpation. The lesion in Paget's disease is rigid, and its edges are hard and

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50. Eller, J. J., and Anderson, N. P.: Cancer Supervention in Skin Diseases: Clinical, Microscopic and Therapeutic Considerations, J. A. M. A. **94**:382, 1930.

51. Kaufmann: Pathology, tr. by Reiman, Philadelphia, P. Blakiston's Son & Company, 1929, vol. 3, p. 1787.

52. Vignolo-Lutate: Monatsch. f. prakt. Dermat. **45**:21, 1909.

53. Jamieson, W. A.: Diseases of the Skin, ed. 4, Edinburgh, Y. J. Pentland, 1894, p. 537.

54. Masland and Babcock: Internat. M. Mag. **8**:81, 1899.

abrupt. From the dry parts of its surface scales can be scraped in which "Paget's cells" can often be detected by microscopic examination, a feature of value in diagnosis.

A carcinoma that has originated in the nipple or from the deeper parts of the underlying breast, or that has originated diffusely beneath the epidermis of the nipple, may be differentiated from Paget's disease as follows: The tumor is not covered by a horny layer. In the undermined epidermis of the nipple there is no sign of Paget's disease. Clinically, the appearance of the nipple may be exactly like that of Paget's disease; the nipple is flattened, and the disease looks superficial; the lesion is rigid on palpation and has hard abrupt margins. It differs from Paget's disease in that no "Paget's cells" can be detected in the superficial scrapings from its surface. The surface when examined by means of a hand lens is usually covered by small sessile nodules and is not flat as in Paget's disease.

We have met with only one example of carcinoma arising in the sebaceous glands on the surface at the top of the nipple.

It occurred on the left nipple of a widow, aged 52. The lesion measured 0.5 cm. in diameter. It was situated directly in the center of the surface at the top of the nipple, and had been first noticed by the patient six weeks before the breast was removed. The surface of the lesion was hard, and its edges were abrupt. The base was excavated and slightly lower than the surface of the nipple. A slight discharge of blood drew the patient's attention to the lesion. The nipple was freely movable, not retracted and was even almost pedunculated. The lymphatic glands were not affected, nor could any change be detected clinically in the small, atrophied underlying breast. On clinical examination, the disease differed from Paget's disease in one respect: No vacuolated epithelial cells, which are typical of Paget's disease, were discovered in a scraping from the surface.

Beneath the epidermis, on the top of the nipple, along its whole extent, there was a collection of sebaceous glands large enough to justify the term "diffuse sebaceous adenomatosis." The neoplastic epithelial hyperplasia was multicentric in origin and affected the ducts of five sebaceous glands, which were in an uninterrupted row. Epithelial invasion of the surrounding tissues had occurred from all the sites of origin. In one place where the invasion was greatest, the carcinoma had spread into the epidermis above, which had been in parts thrown off. Below the epidermis the carcinomatous cells were infiltrating the subjacent tissues from all five sites of origin. The lymphatic vessels were not invaded. The appearances were those of locally malignant tumors. There was no Paget's disease. In only one mammary duct, as it opened onto the surface, and for a little distance below, was there any change, and here it was in a state of desquamative epithelial hyperplasia. In some terminal ducts and their acini there was epithelial neoplasia that looked malignant, but that had not transgressed normal boundaries. Hyperplasia elastica had occurred in a marked degree throughout the whole breast. The subepithelial tissue had undergone diffuse hyperplasia, which in some parts was in a state of hyaline degeneration. There was some infiltration of the subepidermis by lymphocytes and plasma cells. The axillary lymphatic glands were free from carcinoma.

The absence of vacuolated cells was the only reason for excluding a diagnosis of Paget's disease of the nipple. The precise nature of the carcinoma was determined only by microscopic examination. This specimen affords another example of that interesting condition in which a superficial carcinoma is accompanied by an epithelial neoplasia of malignant appearance in the terminal ducts and acini, with no signs of intervening disease.

Syphilis of the nipple may be difficult to differentiate from Paget's disease. It manifests itself either in the early or the late stages. When the nipple is the primary seat of syphilis, it may, on inspection and palpation, closely resemble Paget's disease of the nipple. We have seen two cases in which on incomplete examination of this kind a primary lesion was diagnosed as Paget's disease of the nipple. In one case the true identity of the lesion was discovered only by the demonstration of spirochetes in the specimen after removal of the breast, and in the other, by a positive Wassermann reaction. In the second case, there was no retraction of the nipple. A third example is that afforded by a late syphilitic lesion.

The lesion measured 4 cm. in diameter. The appearance of the nipple was remarkable. Half of its structure had disappeared. The remaining portion was not retracted in the slightest degree, but stood up in its normal position, and its diseased, lateral aspect looked as if the nipple had been divided by a sharp knife and half of it removed. The ulcer on the surface of the areola and nipple was superficial and was more or less rapidly getting larger. The base and the edge of the surface were pliable. The lymphatic glands in the axilla were enlarged and confluent. The disease was of six weeks' duration and had begun at the angle of the junction between nipple and areola. The clinical history of syphilis was clear and unmistakable, and the ulceration disappeared rapidly under the administration of perchloride of mercury and potassium iodide. The disease was diagnosed and treated before the days of the Wassermann reaction.

In the third case, the history of the patient and the clinical signs were sufficiently characteristic to enable the diagnosis of syphilis to be made. The patients suffering from primary syphilis of the nipple did not have a history and clinical signs so distinctive, and an erroneous diagnosis of Paget's disease had been made. A more careful examination in these two cases would have established a correct diagnosis.

A careful correlation between the duration of the disease and the size of the lesion is an important point in the differential diagnosis. In syphilis of the nipple a short duration of the disease is associated with a comparatively large lesion. On the other hand, in Paget's disease the progress is much slower, and a small lesion may be associated with a long history of its presence. Retraction or atrophy of the nipple usually accompanies Paget's disease, but not syphilis. Finally, in the primary syphilitic lesion spirochetes may be discovered, unless antiseptics

have been previously applied, and the Wassermann reaction, which at first is negative, soon becomes positive. The detection of Paget's cells in scrapings from the lesion may help to establish a correct diagnosis. In a suspected syphilitic lesion the therapeutic test may establish the diagnosis.

Paget's disease must be differentiated from diffuse, chronic, subepithelial, possibly infective lesions of the nipple. One of us has examined pathologically two breasts which had been radically removed on the assumption that there was Paget's disease of the nipple. Complete pathologic examination of the nipple and breasts revealed no microscopic sign of this disease. The pathologic states were the same in both instances. The epidermis here and there was in a slightly papillomatous state. The subepithelial tissue of the whole nipple and areola was full of plasma cells, lymphocytes and scattered polymorphonuclear leukocytes. The fibrous tissue elements were also undergoing slight hyperplasia, accompanied by an increase in the supply of capillaries, and in these parts presented the appearances accompanying Paget's disease of the nipple, but as stated, there was no Paget's disease of the nipple.

*Differential Diagnosis.*—When the breast presents the classic clinical signs of Paget's disease on the surface, a correct and decisive diagnosis can be made only by microscopic examination of the whole gland. As so many different interpretations have been made of the morphologic changes that may occur, it would be well to state to which particular changes we are inclined to think the term "Paget's disease of the nipple" should be limited. The usual pathologic events which seem to us to be the most typical of the disease, and to which we should be inclined to limit the term, are as follows: 1. "Paget's cells" are being formed in epithelial cells of the epidermis and openings of ducts. 2. Malignant-looking epithelial neoplasia occurs in the epidermis of these structures and in the cavities of the openings of ducts. 3. This epithelial neoplasia may or may not be in direct continuity with epithelial neoplasia of malignant appearance in the upper reaches of the mammary ducts that terminate in the affected openings of ducts. 4. In the deeper parts of the breast there is either a highly suspicious epithelial neoplasia still confined within normal boundaries or carcinoma that may or may not be clinically obvious.

The inclusion of all these combined pathologic events under the same term is important, because they usually exist in the same breast when the superficial clinical signs of the disease are present. In making this statement we are bearing in mind that the essential clinical features of the disease are caused either by the changes in the epidermis of the nipple and that of the openings of ducts or more rarely by changes in the epidermis of the nipple only.

The temptation to limit the term to those changes that are essential to cause the superficial clinical signs is a great one; it is all the greater because Paget's disease of the nipple affects other parts of the body where there are no mammary ducts and acini to complicate matters. The disease as it affects the nipple is usually accompanied by changes in the ducts and acini. Their omission would leave out features which some authors consider are essential to cause the clinical signs on the surface.

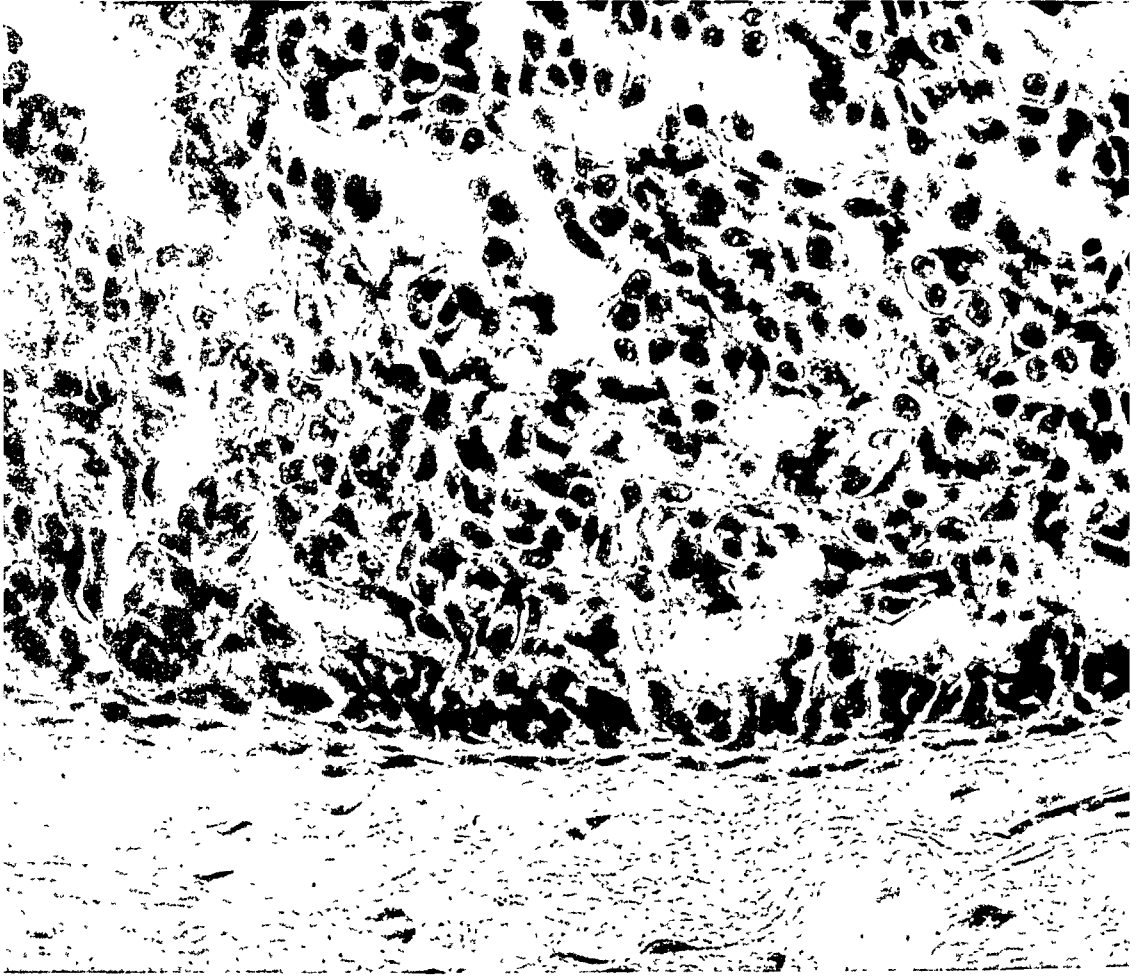


Fig. 9.—High power photomicrograph of figure 8 in which the morphologic appearances seem to indicate that the epithelial neoplasia is arising from the epithelium lining the duct. It certainly looks malignant, although at this point confined within normal boundaries.

*Treatment.*—In the detailed and critical examination of our cases, we have adduced the fact that in six cases the clinical evidence was clear enough to establish the existence of carcinoma in the underlying breast. In ten cases carcinoma existed either in the upper regions only or in the deeper parts of the gland as well, although there was no clinical evidence of its presence, and its discovery was attained only by

careful and methodic microscopic examination of whole sections of these breasts. In the eleventh case there existed in terminal ducts and acini only a neoplastic epithelial hyperplasia which was confined within normal, but distended, boundaries. In this example there was no other sign of epithelial neoplasia either in the nipple or in any opening of a duct. This, therefore, is the only case in the series in which negative clinical signs in the breast were not accompanied by carcinoma, but even

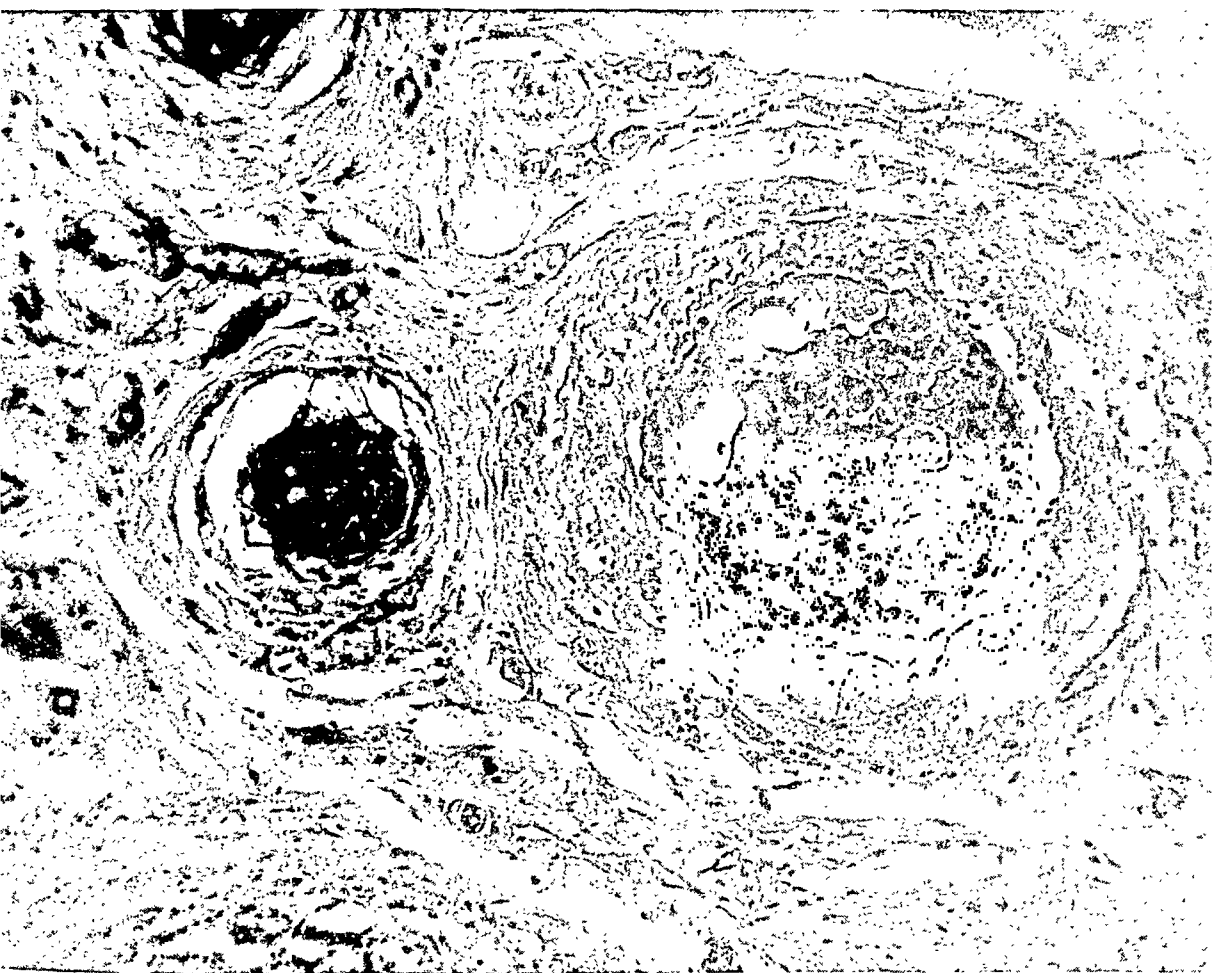


Fig. 10.—Epithelial neoplasia of malignant appearance in terminal ducts and acini of breast presenting Paget's disease of the nipple. There was no clinical evidence of disease in this breast. There was no direct continuity between the carcinoma in the outlet of the duct and that in the acini, in which the carcinoma appears to have arisen spontaneously.

here there was a malignant-looking neoplasia that was still confined within normal boundaries. From this evidence there can be no doubt that the treatment for Paget's disease of the nipple is incomplete and inadequate unless it includes treatment for the condition in the underlying breast, and on this preliminary statement of fact we base the rules which dominate the treatment for Paget's disease of the nipple.



The temptation to adopt conservative measures in the treatment for Paget's disease of the nipple occurs only in those cases in which clinical signs of disease in the underlying breast are absent. Our experience convinces us that this inclination is dangerous and contrary to the results of pathologic investigation and clinical evidence subsequent to conservative measures. Even when the disease on the surface of the nipple is early and extremely small, it is only by microscopic examination of the excised breast that the absence of carcinoma can be established. Even under these circumstances the apparently normal underlying breast should be treated as if it contained carcinoma, because the epithelial neoplasia confined within and distending normal boundaries may exist in terminal ducts and acini, and its future behavior is too problematic to be disregarded. Directly, carcinoma exists in a breast the presence or absence of carcinoma in the axillary lymphatic glands is a matter of doubt, whether they are or are not enlarged. The treatment, therefore, at present lies between the total excision of the breast and its accessible lymphatic system and radiation therapy.

There are two conservative measures in the treatment for Paget's disease of the nipple: (1) removal of the surface disease only and (2) local mastectomy without removing the pectoral muscles or lymphatic glands in the axilla. With regard to the first, it is obvious from pathologic investigations that in spite of the innocent appearance of the lesion on the surface of the nipple, the epithelium in the ducts and acini of the underlying breast exhibits malignant neoplastic changes with such regularity that removal of the surface lesion alone is an inadequate and dangerous procedure. Clinical evidence confirms the pathologic observations.

The second conservative method, local mastectomy only, presents a more difficult problem and is open to the following considerations. The ultimate question is: How often are the axillary lymphatic glands carcinomatous when the breast and the axillary lymphatic glands appear normal on clinical examination? In the majority of instances, when the surface lesion is early, any disease in the underlying breast is still confined within normal boundaries. Under these circumstances the axillary lymphatic glands are free from carcinoma. We have never seen an instance in which a neoplastic epithelial hyperplasia, however malignant-looking, confined within normal boundaries was accompanied by carcinomatous lymphatic glands. There is no method, except that of microscopic examination of the breast, of determining whether or not the neoplastic epithelial hyperplasia is still confined within normal boundaries. In a very early case of Paget's disease of the nipple in which the underlying breast and axillary lymphatic glands appear normal on clinical examination, the justification of local mastectomy only must be considered as a possibility. There can be no doubt that in

capable hands the removal of the axillary glands at the same time that the breast is removed does not increase the danger of the operation, and the performance of the complete operation may save the life of a patient by removing the whole of the disease. Thus Bloodgood stated that there is only one operation for Paget's disease of the nipple and that is the radical one. In a lesion of questionable diagnosis he believed that a local mastectomy is an illogical procedure on the grounds that it is too extensive an operation, if the lesion is benign, and too limited, if the lesion is Paget's disease. With this statement we thoroughly agree. In the comprehensive paper in which Bloodgood<sup>14</sup> described Paget's disease of the nipple he convincingly demonstrated the necessity of establishing definitely that the disease of the nipple is Paget's disease before the treatment is ordained. We have mentioned two cases in which breasts were removed on a diagnosis of Paget's disease in which the diagnosis was proved wrong by subsequent microscopic examination. This raises the question of what steps should be taken in dealing with a doubtful case of Paget's disease of the nipple.

The ideal method obviously should be one in which there is no delay, which involves no risk of spreading the disease, if malignant, and which submits a result that is free from doubt. We have pointed out the value of discovering vacuolated cells of Paget's disease in a superficial scraping. When the result of such an examination is negative, the diagnosis is inconclusive. Under the latter circumstance the only means of diagnosis available is that of microscopic examination of a section. To remove the lesion and embed it in paraffin takes time and exposes the patient to the risk of dissemination of the disease. Is there then any other method that can be adopted? There can be no doubt that when frozen sections are properly made as a preliminary step to an immediate operation, their evidence can be almost as perfect as that of sections embedded in paraffin.

Irradiation of the surface of the nipple showing Paget's disease can accomplish a cure of this part. Examples of such cures are recorded by Haret,<sup>55</sup> Hartzell<sup>34</sup> and others. The guiding principles in the treatment for the surface lesion of Paget's disease are essentially the same as those in the treatment for rodent ulcer, squamous epithelioma, etc. We have pointed out the fallacy of treating the patient for the lesion on the surface alone and disregarding treatment for disease in the underlying breast. In irradiating breasts showing Paget's disease of the nipple the treatment must be aimed, not at the surface lesion alone, but at the disease in the underlying breast. This statement

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55. Haret, G.: Quelques cas de maladie de Paget traités par la radiothérapie, *J. de radiol. et électrol.* 3:416, 1918-1919.

applies to those examples in which clinical signs of carcinoma of the breast are absent, as well as to those in which clinical signs are apparent. Failure to treat adequately the underlying breast has resulted in the healing of the superficial disease and the subsequent development of carcinoma in the subjacent breast. An example of this kind is reported by Dobkevitch, Moulonguet and Nahan.<sup>56</sup> The recurrence of the disease in the underlying breast five years after irradiation is attributed by the authors to the inadequacy of the irradiation of the underlying breast.

Because of the radioresistance of the epithelial neoplasia in the breast underlying Paget's disease of the nipple it is highly questionable whether even a maximum amount of external irradiation is adequate to sterilize the neoplastic process. The difficulty of distributing foci of radium uniformly throughout the gland renders this method of irradiation also somewhat uncertain.

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56. Dobkevitch, S.; Moulonguet, P., and Nahan: *Étude de la maladie de Paget du sein*, Thesis, Paris, 1926.

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, etc.**—Richard W. Linton has resigned as assistant professor of bacteriology in Columbia University in order to go to India to work on cholera under the auspices of the India Research Fund Association.

Charles W. Stiles, chief of division of zoology in the National Institute of Health, United States Public Health Service, whose work on hookworm disease led to the present efforts at its eradication, will go on the retired list Oct. 1, 1931, after having completed forty years of active service for the government.

Col. Charles F. Craig will be retired from the medical corps of the army, at his own request, after thirty-three years of service, and next fall will take up the duties of professor of tropical medicine in Tulane University, New Orleans.

Arthur F. Coca, professor of immunology in Cornell University Medical College, New York, has been appointed clinical professor of medicine in the New York Post-Graduate Medical School. On October 1, next, Dr. Coca will become medical director of the Lederle Laboratories.

Earl Baldwin McKinley, professor of bacteriology in Columbia University and director of the school of tropical medicine at Porto Rico, has been appointed dean of the school of medicine of George Washington University, Washington, D. C.

Hans Zinsser and David Marine have been given the degree of doctor of science, and James Ewing the degree of doctor of laws, by Western Reserve University. Eugene L. Opie has received the degree of doctor of science from Yale University.

A. T. MacConkey, for many years head of the serum department of the Lister Institute in London and known to bacteriologists for his bile salt medium, has died at the age of 70.

Aristide Agramonte, the last survivor of the United States Army yellow fever commission, has died at the age of 63. Dr. Agramonte graduated in medicine from the College of Physicians and Surgeons of Columbia University; he was professor of bacteriology in the University of Havana from 1900 until recently when he resigned and went to New Orleans to organize a department of tropical diseases in the new medical school of the Louisiana State University.

**Grant for Study of Renal Function.**—It is reported that \$100,400 has been appropriated by the Commonwealth Fund for study of the renal function by Alfred N. Richards, professor of pharmacology in the University of Pennsylvania, whose method of microscopic examination of the functioning kidney of the frog is well known.

**International Association for Geographic Pathology.**—The Association will hold its first conference in Geneva, Oct. 8 to 10, 1931. The program consists of a symposium on hepatic cirrhosis, including observations and the results of experimental studies collected by the national committees of the Association in twenty-six countries.

**Society News.**—At its recent meeting the American Society of Clinical Pathologists elected the following officers: H. J. Corper, president; William M. Simpson, president-elect; C. J. Bucher, vice-president, and A. S. Giordano, secretary-treasurer. The Ward Burdick prize of the society was given to W. G. Exton.

The Monaco prize of 100,000 francs, awarded every two years by the Academy of Medicine in Paris to aid some French investigator in his work, has been given to Martin R. Veillon of the Pasteur Institute for his work in bacteriology.

It is reported that a Rockefeller grant of \$45,000 has been given to Columbia University in aid of research on the common cold by A. R. Dochez and Yale Kneeland, Jr.

DOCTORATES IN BACTERIOLOGY AND PATHOLOGY  
GRANTED BY AMERICAN UNIVERSITIES, 1930-1931

Clarence J. West and Callie Hull, Research Information Service, National  
Research Council, Washington, D. C.

BACTERIOLOGY

California: Bernard Stauffer Henry, "Studies on Dissociation in the Genus *Brucella*."

Chicago: Walter LeRoy Mallmann, "Studies on Bacillary White Diarrhea: The Dissociation of *Salmonella Pullorum* and Related Species."

Columbia: Mary Wotherspoon Colley, "Stimulation Phenomena in the Growth of Bacteria as Determined by Nephelometry."

Cornell: Henri Louis Berard, "The Relation of Contamination from Certain Sources to the Subsequent Bacterial Development in Milk." Carl Adam Frey, "The Distribution of Acid-Fast Bacteria in Soils." Harriet Mansfield Thomson, "Studies on Saprophytic Acid-Fast Bacteria."

Harvard: Thomas Patrician Hughes, "The Metabolic Requirements of Staphylococci and Certain Other Bacteria."

Illinois: Florence Lydia Evans, "The Effect of Sodium Chloride, Sodium Nitrate and Sodium Nitrite on *Clostridium Botulinum*, *Clostridium Putrificum* and *Clostridium Sporogenes*."

Iowa State College: Merle P. Baker, "Some of the Relationships Among Organisms in Butter Cultures." Herbert Andrew Derby, "Bacteriological Studies on Butter Showing Surface Taint." Charles Shelton McCleskey, "Some Observations on Gas Production by Bacteria." Michael B. Michaelian, "The Volatile Acids Formed from Citric and Lactic Acids by *Streptococcus Citrovorus* and *Streptococcus Paracitrovorus*." Leonard Garnett Thompson, Jr., "The Effects of Various Nitrogen and Phosphorus Compounds on the Growth of *Azotobacter* and the Fixation of Nitrogen." Maurice Wade Yale, "Studies on the *Escherichia-Aerobacter* Group of Bacteria in Dairy Products."

Johns Hopkins: Ottis Rembert Causey, "A Study of the Bacteria Associated with Blow-Flies and the Production of Sterile Larvae." James Douglas Reid, "Specific Disinfection of *Bacillus Pyocyaneus* by the Organic Acids." Sally Hamilton Stabler, "The Electropure Process of Milk Pasteurization."

Maryland: Arthur Kirkland Besley, "The Effect of Ozone on the Vitamin Content of Cod Liver Oil." Wilbur G. Malcolm, "A Comparative Study of the Efficiency of Certain Germicides in the Preservation of Biologics." Daisy Inez Purdy, "A Study of the Bacteriological Changes Produced During the Ageing of Cured Hams."

Minnesota: Paul Clifford Leck, "The Relation of Fatty Acids and Lipoids to Neurotoxins."

New York: Jacob Weinberg, "The Buffer Capacity of Culture Media and of Standard Buffer Solutions."

Northwestern: Francis Dowden Gunn, "Bone Marrow Reactions in Certain Cases of Thrombocytosis: Ultraviolet Light and a Specific Bacterial Infection."

Pennsylvania: Dorothy Spring, "An Inquiry Into Heterothallism in Certain of the More Common Dermatophytes."

Rutgers: Nandor Porges, "The Production of Citric Acid by *Aspergillus Niger*."

St. Louis: William Frederick Lange, "Gram-Staining of the Colon *Bacillus* in Relation to Time and Metabolism."

Vanderbilt: John Y. Sugg, "Diphtheria Antitoxin in Human Saliva."

Western Reserve: Ralph Heeren, "Biological Characteristics of the So-Called Fecal and Soil Types of *Escherichia Coli* and *Communior*."

Wisconsin: William Preston Allyn, "Oxidation-Reduction Potentials in Relation to the Growth of an Aerobic Form of Bacteria." Theron Hervey Butterworth, "A Contribution to the Study of *Lactobacillus Acidophilus*, with Special Reference to a Commercial Concentrate." David Hanon Dunham, "Physiological Relationships Between the Various Strains of Rhizobia and the Leguminosae." Charles Axtell Hunter, "The Dissociation of *Escherichia Coli*." Charles Homer Keipper, "A Study of the Number and Distribution of Micro-Organisms on Unwashed and Washed Cabbage." David Gordon Laird, "Bacteriophage and the Root-Nodule Bacteria."

Yale: Ezra Philip Casman, "The Limitation of Bacterial Growth at Higher Temperatures." Carroll Walter Grant, "The Mucosus Capsulatus Group of Bacteria." John Harold Hanks, "The Toxin Production of *Bacterium Pullorum* and the Role of This Toxin in Pullorum Disease." Louis Weinstein, "Some Factors Involved in the Biological Production of Acetone and Butyl Alcohol."

#### PATHIOLOGY

Chicago: Arthur John Vorwald, "The Cellular Reaction to Infection with Tubercle Bacilli in Animals of Varying Resistance."

Harvard: Hsin Tao Chen, "Host Reactions of *Ctenocephalus Felis* to *Dipylidium Caninum*."

Johns Hopkins: Herald Rea Cox, "Physical Factors Involved in Ultrafiltration." Gordon Ernest Davis, "Complement Fixation in Experimental Yellow Fever in *Macacus Rhesus* Monkeys." Dennis Daniel Donahue, "The Effect of Radiation on the Resistance of Chickens to Fowl Cholera." Paris Emancipacion Menendez, "Serological Relationships of *Entamoeba Histolytica*." John Edward Stumberg, "Immunological Aspects of Hook-Worm Disease." Teodulo Topacio, "The Behavior of Rabbit Virus III in Tissue Culture." Randall Leslie Thompson, "Electrophoresis of Bacteria; the Diphtheria and Pneumococcus Groups."

Michigan: Richard Ellsworth Olsen, "A Study of the Granular and Atypical Forms of *Spirochaeta Pallida* in Tissues."

Northwestern: Stuart L. Vaughan, "Bone Marrow Reactions: The Leukocyte Response in Man to the Toxic Filtrate of Scarlet Fever *Streptococcus* (Dick)."

Purdue: Loyal Witherow Fisher, "Studies on Hemolytic *Streptococci* Isolated from Cases of Hemorrhagic Smallpox."

Wisconsin: Agustin Rodolfo, "The Permeability of the Placenta to Antibodies and Its Relation to Serology as a Factor of Orthogenetic Change."

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

A SYNDROME CHARACTERIZED BY CONGENITAL CLOUDING OF THE CORNEA AND BY OTHER ANOMALIES. HENRY F. HELMHOLZ and ETHEL R. HARRINGTON, *Am. J. Dis. Child.* **41**:793, 1931.

The characteristics of the syndrome are: uniform cloudiness of the cornea; restricted motion of the joints of the extremities; short, thick, clawlike hands and feet, with limited extension; lumbar kyphosis; scaphocephalic head and mental retardation.

AUTHORS' SUMMARY.

THE EFFECT OF THE INJECTION OF URINE FROM PREGNANT MAMMALS ON OVULATION IN THE RABBIT. F. F. SNYDER and G. B. WISLOCKI, *Bull. Johns Hopkins Hosp.* **48**:362, 1931.

The urine of various pregnant animals (macaques, rabbits, rats, cats and dogs) does not produce ovulation in mature, healthy, isolated rabbits. The ovulation test in the rabbit does not completely parallel the similar test in the mouse as reported in the literature. The urine of pregnant monkeys and anthropoid apes has been described as giving positive reactions in the mouse. In our experiments the urine from pregnant macaques has given negative reactions in the rabbit. Of the two tests available for the diagnosis of pregnancy, the ovulation test in the rabbit appears to be more specific than the mouse test. The observation that human urine of pregnancy produces ovulation in mature rabbits has been confirmed.

AUTHORS' SUMMARY.

EXPERIMENTAL NEPHRITIS IN THE FROG. J. OLIVER and E. SHEVSKY, *J. Exper. Med.* **53**:763, 1931.

A method of testing the frog's kidney by means of perfusion is described. This is made possible by dissociating, as far as possible, from the total function of the organ the functions of its constituent parts. The characteristics by which tubular, glomerular and combined tubular-glomerular lesions may be recognized are described.

AUTHORS' SUMMARY.

EXPERIMENTAL NEPHRITIS IN THE FROG. J. OLIVER and P. SMITH, *J. Exper. Med.* **53**:785, 1931.

It is possible to produce in the perfused frog's kidney an experimental nephritis that is anatomically similar to the nephritis that develops in the living animal. The functional effects of these anatomic alterations may be examined by a method previously described. The correlation of the two aspects of damage, anatomic and functional, is more certain under such conditions than in the living animal. The value of the extravital method in general problems is indicated by our brief consideration of mitochondrial changes.

AUTHORS' SUMMARY.

HYPERSENSITIVENESS TO SOLUBLE SPECIFIC SUBSTANCES FROM YEAST-LIKE FUNGI. H. D. KESTEN and E. MOTT, *J. Exper. Med.* **53**:803 and 815, 1931.

The polysaccharide fractions from each of five yeastlike fungi produce rapid, fatal anaphylactic shock in guinea-pigs passively sensitized with antiserum from rabbits immunized against the killed organisms. Cross-anaphylactic reactions with

heterologous polysaccharide fractions are frequent. They parallel closely the cross-precipitin reactions, thus adding evidence in favor of the identity of precipitin and sensitizing antibody. The polysaccharide fraction from *Monilia psilosis* produces anaphylactic death in guinea-pigs actively sensitized with killed homologous organisms, but an attempt to sensitize actively with the polysaccharide fraction was unsuccessful.

The anterior chamber of the rabbit eye was sensitized by the local injection of heat-killed *Monilia psilosis*. Subsequent intravenous injection of a polysaccharide fraction prepared from the same organism elicited a reaction in the sensitized eye in five of twelve rabbits.

AUTHORS' SUMMARIES.

THE INFLUENCE OF X-RAY LESIONS OF THE INTESTINAL MUCOSA ON ABSORPTION OF GLUCOSE AND OTHER SUGARS. K. W. BUCHWALD, *J. Exper. Med.* **53**:827, 1931.

A sublethal dose of x-ray was applied over the abdomen of rats and over the thorax for control purposes. Twenty and forty hours after radiation (i. e., during the "latent period") the rate of absorption of dextrose, fructose and mannose was markedly diminished. Simultaneously definite histologic changes were observed in the intestinal mucosa. In spite of the decrease in the absolute amount absorbed, the relative rate of absorption of the three sugars mentioned remained nearly unchanged, indicating that the epithelial cells retained their selective action on sugars.

AUTHOR'S SUMMARY.

EXPERIMENTAL HYPERTHYROIDISM IN GUINEA-PIGS AND RABBITS. G. RAKE and D. McEACHERN, *J. Exper. Med.* **54**:23, 1931.

A study has been made of the pathologic changes in the hearts and other tissues of animals in which hyperthyroidism was caused with thyroxine. Forty-four rabbits and seventeen guinea-pigs were given intramuscular injections of thyroxine every other day and killed at varying intervals. Tissues from a series of normal animals (twenty guinea-pigs and forty-three rabbits) were examined as a control. The changes in the heart and other tissues of animals with hyperthyroidism were insignificant and varied but little from changes seen in normal control animals. Of eight thyrotoxic guinea-pigs that developed coincidental infection (bronchisepticus) all showed myocardial lesions. Of nine thyrotoxic guinea-pigs, free of infection, only one gave evidence of myocardial change. It is pointed out that hyperthyroidism, per se, cannot be held responsible for these lesions, which would appear to have been associated with the infection. It was noted that rigor mortis of the skeletal muscles occurred much sooner in the bodies of animals with hyperthyroidism than in normal animals.

AUTHORS' SUMMARY.

EFFECT ON PULMONARY EPITHELIUM BY INTRAPLEURAL INJECTIONS OF ELECTROLYTES. J. S. YOUNG, *J. Path. & Bact.* **34**:357, 1931.

Hyperplasia of the epithelium lining the marginal alveoli of the lung of the rabbit can be produced by a single intrapleural injection of a three quarters-normal solution of strontium chloride. A second injection of the same solution within fifteen or twenty days of the first fails to produce a further reaction but at a longer interval it becomes effective again. Provided that the concentration of the reagent is gradually increased in successive injections, commencing with a weak solution (e. g., sixteenth-normal), intercurrent hyperplasia can be prevented, and finally no reaction is produced by a test injection of a normal solution which will occasion a vigorous reaction in normal control animals. The incidence of hyperplasia affords a general but reasonably accurate criterion of the susceptibility of the epithelial cells to certain stimuli of the nature of graduated solutions of electrolytes. The resistance of the cells can be measured arbitrarily by that con-



centration of strontium chloride or other electrolytes which just fails to produce a reaction. According to this standard, the normal resistance is represented by a half normal solution of strontium chloride although it is subject to a minor degree of variation. As the result of previous treatment, however, the cells can acquire an increased resistance corresponding to a normal solution of the same salt. This increased resistance is a local phenomenon; it cannot be established by intravenous or by intraperitoneal injection of the electrolyte. Second, it can be produced either by a single massive intrapleural injection which is associated with a vigorous epithelial reaction, or, alternatively, by a series of weaker injections gradually increasing in concentration to a maximum without any intercurrent reaction. Third, it is not strictly specific since it would appear that calcium chloride can afford some measure of protection against strontium chloride; on the other hand, the resistance is readily broken by trauma or by the disintegration products of red blood corpuscles. The significance of these observations is discussed with reference to the nature of the biologic processes concerned in the onset of proliferation and it is concluded that they are consistent with the hypothesis that cell division is initiated by a precipitation of the colloids of the cell membrane.

#### AUTHOR'S SUMMARY.

THE EFFECT OF TESTICULAR EXTRACT UPON TISSUE PERMEABILITY. D. McLEAN, *J. Path. & Bact.* **34**:459, 1931.

It has not yet been possible to isolate the active substance in extracts of testicle. These extracts may be sterilized without loss of activity by saturation with chloroform, and the alcohol ether precipitate may be sterilized by dry heat at 160 C. for thirty minutes. The permeability of skin removed from the killed animal is increased by the extract for at least forty-eight hours after death. Skin that has been removed, desiccated and then soaked in water will still show increased permeation by the extract. The extract causes swelling and distortion of the fiber bundles of the dermis. The appearance and significance of this change is discussed. A similar effect is produced by protamine salts. A relation between the surface tension of the extract and the diffusibility could not be demonstrated, nor was there increased diffusion in agar, gelatin or blotting paper. Extracts of spermatozoa possess the same activity as extracts of whole testicle. Preliminary observations indicate that these extracts increase the permeability of ova. The significance of these observations is discussed. Trypsin destroys the activity of the extract. Peptic digestion is resisted at the lowest  $pH$  at which it is possible to work with this extract. Though there is some peptic activity at this  $pH$ , digestion is not complete. Clupeine sulphate, a characteristic protamine salt, which increases dermal permeability, but inhibits vaccinal lesions in the skin, inhibits the growth of *B. typhosus* in culture.

#### AUTHOR'S SUMMARY.

MITOGENETIC RADIATION. S. B. SEWERTZOVA, *Ann. Inst. Pasteur* **46**:337, 1931.

The influence of radiation from tissue on cell division was approached experimentally by a technic involving the exposure of each of four bacterial species to (1) a fresh yeast culture, (2) a heart in rhythmic contraction, (3) tetanized frog muscle and (4) the spleen of a frog. Bacteria, multiplying rapidly and easy to follow quantitatively, were considered preferable to yeast cells or to vegetable root cells wherein mitoses might be watched. Bacterial division was increased by exposure to tetanized muscle by 76.82 per cent; to heart, 38.4 per cent; to spleen, 32.5 per cent, and to yeast, 21.9 per cent. Control tests gave these figures significant weight. The periods of exposure were varied between a few minutes and some hours. Exposures were made through a quartz plate, half of which was covered with lead for a control chamber, at a distance of slightly more than 13 mm.

M. S. MARSHALL.

## PLANT AND ANIMAL EXPERIMENTS WITH A SHORT WAVE RADIO OSCILLATOR.

K. v. OETTINGEN, *Strahlentherapie* 41:251, 1931.

A short wave radio oscillator with a wavelength of 3 meters was used. The waves act on inorganic and organic, dead and living matter with the production of heat. But the maximum heat effects occur at different location as those caused by diathermy. Mice brought into the condensator field become first restless. After the appearance of condensed water on the walls of the glass container they show listlessness and increased respiratory activity, followed by paresis of the hind legs. The animals are then highly sensitive against noises. If the experiment is stopped at this stage, the animal may recover with the loss of the tail, ear or legs by dry gangrene. Continuation of the treatment results in the appearance of clonic spasms, which gradually involve the whole body and become tonic in character. Death can be caused in two minutes with strong doses. The rectal temperature at this time is 43 C., and the body is rigid. Careless handling of the apparatus by the attendants may result in the appearance of dizziness, headache, nightly attacks of fear, increase in body temperature by several tenths degrees, general malaise, albuminuria and disturbances of libido sexualis. Exposure of the hands to the waves is followed by the appearance of a vibratory feeling which gradually changes into a feeling of a deeply seated, painful heat, leaving after a few hours a feeling of weakness. All these symptoms disappear after a few days. Metals are not heated by the waves. Organic substances, like bread; plants, etc., may be heated to the degree of carbonization or even burning with fire. Electrolyte solutions, oils, especially turpentine oil, are heated. The heating effect does not depend on the atomic, molecular or specific weight or the  $p_H$  of the solution. The dielectric constant and the conductivity are apparently the most important factors in this respect. While fat tissue, bones and skin are mainly heated by diathermy, skin and fat tissue are least heated by short radio waves. Blood, especially hemolyzed blood diluted 50 per cent with water, is strongly heated, while serum behaves like physiologic solution of sodium chloride with a moderate heat production. As the heating effect depends on the electrolyte concentration, the type of electrolyte and the wavelength, different tissues vary in their reactivity, as for instance normal tissue from tumor tissue. Turpentine oil can be polymerized by radio waves in a few minutes, a process which takes many hours if done by simple heating. Beside the direct heating and electric effects, also indirect, injurious effects on distant organs have to be considered. Small doses stimulate plant growth and sugar fermentation by yeast. Radio waves have no effect on bacterial growth (*streptococcus*, *gonococcus*, *meningococcus*, *pneumococcus*, *tubercle bacillus*, *Bacillus prodigiosus*). Mice given intraperitoneal injections of pneumococci or fed with *B. enteritidis* Breslau showed a prolongation of life after irradiation, but on account of the unavoidable total irradiation of the mice generalized injurious effects could not be excluded. Beneficial effect is believed to be due to mobilization of defense forces. Eggs of frogs, toads and axolotls irradiated with the radio waves caused the death of the ova if large doses were used, while the application of moderate doses resulted in the production of malformations with lowered vitality. Small doses caused an acceleration in the development of toad's eggs. Toad's eggs were more resistant to the radio waves than frog's eggs. Irradiated rabbits developed first a leukopenia of the blood due to a decrease of the lymphocytes accompanied by an increase of the pseudo-eosinophils, followed by a leukocytosis with an increase of lymphocytes and decrease of the pseudo-eosinophilic cells. The coagulation time and sedimentation time were decreased. Mice rapidly killed by large doses showed at autopsy a generalized hyperemia with superficial hemorrhages beneath the serous membranes, edema of gastric and intestinal mucosa and a peculiar whitish edema of the muscle tissue. The microscopic examination of the organs showed hemorrhages, atelectasis and vicarious emphysema in the lungs, hemorrhages in the liver, hyperemia and nephrosis in the kidneys, vacuolization of the glia in the hemispheres of the brain, shrinkage of cytoplasm of the tubular epithelium of testicle, hyperemia in ovary and vacuolization of the sarcoplasm of the muscle tissue. Mice treated

over a prolonged period with small doses did not show any characteristic organic changes at autopsy, but the histologic examination of the organs revealed definite injurious organic effects, such as hemorrhages into the lungs, proliferation and giant cell formation around hemorrhagic foci in the liver, fibrin clots in the hepatic veins, nephritic changes in the kidneys, degeneration of nerve cells and vacuolization of glia cells in the brain with encephalitic foci in the cerebrum and cerebellum, perivascular lymphocytic infiltrations in the meningi, degeneration and necrosis of testicular epithelium, disturbed spermiogenesis, degeneration of ova and granulosa cells in the ovary with subsequent regenerative changes in this organ. During the first weeks after irradiation treatment estrus was more frequent. Libido was preserved, pregnancy occurred and resulted in normal litters. Irradiation of cancer in mice produced usually a regression of the tumors, but occasionally was followed by very rapid growth.

WILHELM C. HUEPER.

**RADON EFFECTS UPON THE BLOOD SUGAR AND ITS DISTRIBUTION BETWEEN CELLS AND PLASMA.** N. KOTSCHNEFF, *Strahlentherapie* 41:359, 1931.

During starvation the liver releases sugar into the blood of the liver vein. The amount of sugar released can be calculated from the blood sugar in the blood of the portal vein and that in the hepatic vein. Different organs retain unequal amounts of sugar from the arterial blood. Venous blood therefore contains less sugar than arterial blood. The largest amount of sugar is retained by the intestine and muscle. Therefore, portal and femoral vein contain the smallest amount of sugar during starvation, while the portal venous blood contains the highest amount of sugar during digestion. Sodium fluoride was used as an anticoagulant on account of its glycolysis inhibiting action in the experiments. The introduction of radon into the gastro-intestinal tract during carbohydrate digestion causes a marked increase of the blood sugar in all vessels. This is due to a decreased sugar retention by the liver and muscle and an increased retention of it by the kidney as demonstrated on angiotomized dogs and rabbits in which blood was withdrawn through cannulas introduced into the portal, hepatic and renal veins. Also, during starvation sugar retention by the kidney was increased if radon was injected into the blood or intestine. The resorption of sugar was not essentially altered by the radon medication. The increase of blood sugar is due to an increase of this constituent in the plasma, while the cellular sugar of the blood is in all vessels relatively decreased. During starvation, the liver, kidneys and intestine retain less plasma sugar and more cellular sugar if radon is given. During normal carbohydrate digestion, relatively more sugar is retained by the blood corpuscles than by the plasma. Radon medication during this period causes a general hyperglycemia, an increased retention of the plasma sugar by the intestine and a decreased retention of the cellular sugar, resulting in a decreased sugar retention in the liver, because the liver releases during the period, as always, only plasma sugar into the blood, retaining only the cellular sugar. Also the kidneys retain under these conditions much more cellular sugar than plasma sugar.

WILHELM C. HUEPER.

### Pathologic Anatomy

**FATAL ACUTE LYMPHOBLASTIC LEUKEMIA IN AN INFANT.** PARK J. WHITE and EDWARD L. BURNS, *Am. J. Dis. Child.* 41:866, 1931.

In the light of the observation of others, the case herewith reported is of interest chiefly because the severe, lymphoblastic, febrile leukemia, with prolonged clotting time, and with extreme lymphoblastic infiltration of both kidneys, apparently had its origin on the fourth day of life, when the first "leukemic spot" appeared. Clinically, the nature of the enlargement of the kidneys was, of course, in doubt until the observations on the blood were reported.

AUTHORS' SUMMARY.

FUNCTIONALLY TWO-CHAMBERED HEART. L. MINOR BLACKFORD and LEWIS D. HOPPE, *Am. J. Dis. Child.* **41**:1111, 1931.

In an infant, aged 6½ months, the right atrium opened through the foramen primum into the left atrium, which in turn opened through the mitral valve into a single ventricle. The tricuspid valve was absent. The aortic valve was normal; the pulmonic, bicuspid. Extreme stenosis of the ventriculobulbar junction prevented the passage of an adequate amount of blood to the lungs. A theory is advanced to explain the pathogenesis. More detailed dissection revealed a small right ventricle in the mass of ventricular muscle, between the stenosed opening in the anterior wall of the large ventricle, and a second stenosis at the beginning of the bulbus cordis. In other words, an interventricular septum was present, although incomplete. Functionally, however, we can still state that the heart was two-chambered.

AUTHORS' SUMMARY.

NONRACHITIC SOFT CHEST AND FLAT HEAD, A NEW SYNDROME. A. F. HESS, *Am. J. Dis. Child.* **41**:1309, 1931.

A clinical condition previously termed by me "nonrachitic softening of the ribs" has been made the subject of further study. It has been found that this abnormal softening results in the sinking or flattening of the wall of the chest, which is a frequent occurrence in children, and which may persist into adult life. In association with this pathologic condition of the thorax, a pronounced flattening of the occiput was often noted. This malformation is due to pressure and may persist for many years. Bowing of the legs and flatfoot are sometimes associated with these deformities. These lesions of the bones are not of rachitic origin. In the cases to which I refer, there were none of the typical signs of rickets; at all times the roentgenographic picture did not show any abnormality, and examination of the blood showed that the phosphorus and calcium concentrations were normal. Histologic examination did not show rickets at the costochondral junctions in two cases in which postmortem examinations could be made. Furthermore, the softening of the chest did not respond to any of the specific antirachitic agents or to the established vitamins. The syndrome—flat head and soft chest—does not belong in the category of rickets and should be differentiated from this disorder. It is a form of osteoporosis that may be of congenital origin.

AUTHOR'S SUMMARY.

ASYMMETRY OF THE HEAD AND FACE IN INFANTS AND IN CHILDREN. D. GREENE, *Am. J. Dis. Child.* **41**:1317, 1931.

Asymmetry of the occipital region is a common condition in infancy. This asymmetry is found to be accompanied by an associated asymmetry of the face, leading to an increased height of the cheek bone and a difference in the level of the ears. At times it results also in an irregularity of the dental arches and a lateral deviation of the nose. Although this condition may result from rickets, it is more frequently the result of a nonrachitic condition, an osteoporosis of the bones. The softening of the bones of the head is part of a symptom complex that includes softening of the ribs and of some of the long bones of the body. The deformity of the occiput is due to almost constant pressure on the osteoporotic bones resulting from the posture of the infant. This asymmetry of the head and face can be corrected in the early months of life by merely so changing the posture of the infant that the pressure falls on the opposite side of the head. This preventive measure is worth while, as the deformity frequently persists into childhood, and in some cases is permanent.

AUTHOR'S SUMMARY.

CONGENITAL AIR CYST OF THE LUNG. A. H. PARMELEE and C. W. APFELBACH, *Am. J. Dis. Child.* **41**:1380, 1931.

A congenital air cyst of the lung in a 17 months old infant together with observations made at autopsy is recorded and analyzed. Autopsy findings prove the cyst to be a huge solitary bronchiectatic cavity. The probable etiology is discussed.

AUTHORS' SUMMARY.

DUODENAL OBSTRUCTION CAUSED BY CONGENITAL BANDS. E. F. BURT and R. M. TYSON, *Am. J. Dis. Child.* **41**:1403, 1931.

In these four cases the obstruction was caused primarily by transduodenal bands originating in the undersurface of the liver. This corresponds with the hepato-duodenal ligament described by anatomists and the transduodenal bands described by clinicians as a cause of constriction of the duodenum in adults. Taylor has commented on additional adhesions like those in cases 1 and 4, which he has found in adults. The ligament or band is of congenital origin. The adhesions of the duodenum, pylorus and stomach can be explained only on the grounds of inflammation, possibly as a result of chronic traction during rotation of the intestine, caused by the presence of the accessory ligament. The entire clinical picture closely simulates that of hypertrophic stenosis. The time of onset seems to depend on the denseness of the adhesions. In cases 1 and 4 the bands and adhesions were fairly dense, and symptoms of acute obstruction began immediately at birth. In cases 2 and 3 obstruction became acute in four and sixteen weeks, respectively. The absence of a palpable mass in the abdomen is an important differential point. It is important that a laparotomy be performed as soon as a diagnosis of obstruction is made. The excellent results obtained in cases 1 and 2 can be attributed to operative intervention before the babies were markedly dehydrated and malnourished.

AUTHORS' SUMMARY.

PATENT DUCTUS ARTERIOSUS WITH PRIMARY BACTERIAL PULMONARY ENDARTERITIS. W. H. TRIMBLE and RALPH M. LARSEN, *Am. Heart J.* **6**: 555, 1931.

A case of pulmonary endarteritis, with the vegetation superimposed on the wall at the orifice of a patent ductus arteriosus, which was diagnosed clinically six and a half months before death and confirmed by autopsy, is reported. Although the mitral valve was also the site of vegetations, the pulmonary endarteritis is shown to be the primary lesion.

AUTHORS' SUMMARY.

ACQUIRED RHEUMATIC PULMONIC STENOSIS AND INSUFFICIENCY. SIDNEY P. SCHWARTZ and DAVID SHELLING, *Am. Heart J.* **6**:568, 1931.

The interesting lesion in this patient as revealed by the autopsy findings, in addition to the evident mitral and tricuspid lesions, is an acquired rheumatic valvulitis superimposed on a malformation of the pulmonic valves which in this case consisted of the absence of one of the pulmonic leaflets. This underlying congenital malformation may explain the unusual dilatation of the pulmonic artery for so long a period since the first diagnosis of pulmonic insufficiency was made seven years prior to her death; for it is well known that such congenital defects of this artery may produce an insufficiency of the valves even in the absence of any acquired disease. It is likely, in view of the rarity of acquired rheumatic lesions of the pulmonic artery, that the congenital malformations were responsible in part for the localization of the rheumatic virus on the pulmonic leaflets. The characteristic x-ray picture of pulmonic insufficiency, as described by one of us in a previous communication, was of great help in the diagnosis of that particular lesion, whereas the stenosis of the valves was suspected solely from the clinical evidences

which in the main were the rough systolic thrill and localized murmurs in the region of the second intercostal space to the left of the sternum.

## AUTHORS' SUMMARY.

PROGRESSIVE THROMBOSIS OF THE PULMONARY ARTERY. CLARENCE H. BOSWELL and HAROLD D. PALMER, *Arch. Int. Med.* **47**:799, 1931.

A case of progressive thrombosis of the pulmonary artery, following an acute respiratory infection, which came to autopsy five weeks later, is reported. The symptoms were malaise, dyspnea, anorexia, palpitation, easy fatigue and "some aching through the chest." There were a slight leukocytosis, a slight elevation of the pulse and respiration and a variation of the temperature between 97 and 99 F. A roentgenogram of the chest showed an increased density of the shadow of the right pulmonary artery. Necropsy revealed that the lungs were crepitant and showed no solid areas except for a small hemorrhagic infarction just beneath and including the pleural surface of the right lung. An old firmly attached thrombus was found in the right pulmonary artery. This vessel, as well as the bronchus in contact with it, showed acute inflammation. A fresh, but firmly attached, thrombus was present in the left pulmonary artery. The authors hold this to be a case of thrombo-arteritis, primary in the pulmonary artery and not embolic.

J. N. PATTERSON.

ACUTE ISOLATED MYOCARDITIS. C. E. DE LA CHAPELLE and I. GRAEF, *Arch. Int. Med.* **47**:942, 1931.

A case of so-called isolated myocarditis of unknown etiology is reported. The clinical and necropsy observations are compared with those in previously recorded cases. Electrocardiograms showing evidence of severe impairment of conduction are noted for the first time in connection with this disease. The gross and microscopic observations demonstrate the distribution of the lesion. The diffuse and focal cellular infiltration consisting chiefly of lymphocytes, the new formation of blood vessels and connective tissue, involving both the interstitium and the parenchyma, the well marked deposits of organized connective tissue and the focal areas of necrosis account for the progressive circulatory failure. Careful microscopic studies have not been made of the conducting tissue. There were no vascular, perivascular or myocardial lesions suggesting rheumatic infection, and no Aschoff bodies were found. The necropsy observations adequately account for the clinical course. The etiology in our case remains obscure. Whether or not the lesion was an atypical rheumatic inflammatory reaction cannot be answered. Aschoff felt that the lesions in his case could not be explained or described as rheumatic alone. He suggested that some other etiologic factor was combined or superimposed, possibly an infection of cryptogenic origin. We believe, with Scott and Saphir, that to regard these cases as rheumatic would not aid in clarifying the knowledge of their pathogenesis.

AUTHORS' SUMMARY.

THE APPEARANCE OF HISTIOCYTES IN THE PERIPHERAL BLOOD. WILLIAM DAMESHEK, *Arch. Int. Med.* **47**:968, 1931.

The histiocyte has distinctive histologic and functional characteristics that make its presence in the peripheral blood stream easily recognized. In disorders that involve the reticulo-endothelial system, the histiocytes may appear in the peripheral blood usually coincident with an increase in monocytes. This is analogous to reactions of the bone marrow in which myelocytes appear in the peripheral blood coincident with an increase in polymorphonuclear cells. Histiocytes in the peripheral blood were found especially when monocytosis was present. Thus they were seen in monocytic (histiocytic) leukemia, in agranulocytosis, especially in the phase of recovery, in septicemia, in subacute bacterial endocarditis, in the convalescent stage of rheumatic fever and rheumatic endocarditis, rarely in

tuberculosis and other infections, frequently in dementia paralytica (in one case associated with convulsions they were found in large numbers) and occasionally in miscellaneous conditions, especially in the leukemias and lymphoblastomas. Other authors have described their occurrence in typhoid fever, cholera, malaria, kala-azar and other diseases. It is felt that the histiocyte as seen in the peripheral blood is a distinctive cell not normally present, that it is in intimate relationship and probably the direct precursor of the monocyte, though ordinarily without relationship to the other leukocytes of the blood, and that it appears in the peripheral blood when there is unusual activity, marked irritation or leukemic proliferation of the reticulo-endothelial system.

AUTHOR'S SUMMARY.

THE MORBID ANATOMY OF HEARTS IN HYPERTHYROIDISM. D. McEACHERN and G. RAKE, *Bull. Johns Hopkins Hosp.* 48:273, 1931.

The literature on the pathology of the heart in hyperthyroidism is reviewed, and the contradictions of evidence are pointed out and discussed. Twenty-seven cases of patients dying with hyperthyroidism are reported, with clinical and pathologic notes. The cardiac findings are compared with those in 150 suitably chosen control cases. In fourteen instances the hearts were normal. In eight instances moderate perivascular or intermuscular fibrosis or small round cell infiltration was found; similar changes were also encountered among the control cases, though less frequently. Conspicuous alterations were found in five instances, in three of which there was coexistent heart disease. Cardiac hypertrophy was noted in 16 of the 27 cases. No relationship could be established between the incidence of auricular fibrillation or the duration of hyperthyroidism and the ultimate findings in the heart. Congestive heart failure occurred in 5 of the 6 cases that presented coexisting organic heart disease. From this evidence it is impossible to ascribe the cardiac phenomena of the disease to structural changes in the muscle. It is pointed out that the hearts from hyperthyroid animals continue to beat, when isolated, at a much faster rate than similar preparations from normal animals. Emphasis is placed on the desirability of studying the problem from the point of view of metabolic and functional alterations in the myocardium.

AUTHORS' SUMMARY.

SUBCUTANEOUS NODULES OF THE JUXTA-ARTICULAR TYPE. H. HANFORD HOPKINS, *Bull. Johns Hopkins Hosp.* 49:5, 1931.

The cases of fourteen patients with subcutaneous nodules of the juxta-articular type are reported. In twelve of the patients the nodes were syphilitic and in two they were associated with chronic infectious arthritis. The syphilitic nodes healed completely under antisyphilitic treatment; the nodes associated with chronic infectious arthritis did not. The histopathologic picture of the nodes in syphilitic patients could not be considered conclusively characteristic of syphilis, nor could it be differentiated from the picture of the nodes associated with chronic infectious arthritis. Spirochetes could not be found in the syphilitic nodes, and one rabbit inoculation was negative. Subcutaneous nodules of the juxta-articular type are not pathognomonic of any single disease. Juxta-articular nodules may be simulated by xanthoma.

AUTHOR'S SUMMARY.

OBLITERATIVE PULMONARY ARTERIOSCLEROSIS. W. G. MACCALLUM, *Bull. Johns Hopkins Hosp.* 49:37, 1931.

A colored woman, aged 39, who presented symptoms of dyspnea, cyanosis, cough and enlargement of the heart, especially on the right side, on postmortem examination, was found to have an obliteration of the pulmonary arterioles. This obliteration was the result of a proliferation of large cells of the intima with cytoplasm laden with fat. The media and adventitia showed no gross changes. Hyaline thrombi in varying stages of organization and canalization were frequent. The

veins showed no changes. The author interprets the intimal changes as primary and the thrombi as secondary. Syphilis as the exciting factor is denied. This was the first case that the author had noted in 12,000 autopsies. (See article by Rosenthal, Sol Roy: Sclerosis of Pulmonary Artery and Arterioles, *ARCH. PATH.* 10:717, 1930.)

EXTRADURAL VENTRAL CHONDROMA. C. A. ELSBERG, *Bull. Neurol. Inst., New York* 1:350, 1931.

Some chondromas originate from the posterior border of a vertebral disk and thus are situated in front of the dura (disk chondromas or ventral extradural chondromas). The first patient with this disorder observed by Elsberg was successfully operated on. In the present contribution fourteen more cases are recorded. They consist of the most frequent type of extradural tumors, occurring in middle-aged people, mostly in men. The site is preferably the cervical region. The size is usually small, varying from 1 to 2 cm. in the greatest length, ordinarily about 1 cm. wide, and seldom more than from 0.5 to 1 cm. in thickness. The cartilaginous nodules observed by Andrae and Schmorl, mostly in women, are considered by Elsberg as possible rudimentary chondromas.

Microscopically, the ventral chondromas were identical with the structure of normal intervertebral disks, the difference being rather of degree than of kind. The majority of the small growths arising from the posterior edges of the intervertebral cartilages are not neoplasms but ecchondromas, local hyperplasias of the cartilage. The tumors can be removed completely and generally give a good prognosis.

GEORGE B. HASSIN.

MORBID ANATOMY AND PATHOGENESIS OF LEPROSY. P. P. DWIJKOFF, Frankfurt. *Ztschr. f. Path.* 40:185, 1930.

A case of leprosy of fourteen years' duration is described. The anatomic diagnosis was tuberculous form of leprosy, ulcerations of the tongue, pharynx, larynx, trachea and upper and lower extremities, gangrene of the toes, and scars over the entire body. There were a healed tuberculosis of both lungs and amyloid infiltration in some organs. Sections of the skin showed that the epithelium was thinned out, the papillary arrangement of the corium being not clearly recognizable. Below the epidermis there was a new formation of connective tissue and elastic fibers, forming a wall surrounding the deeper lesions, which is referred to as "protective wall." Below this wall there were many fields showing cells that contained an abundance of vacuoles within their cytoplasm. These cells varied in size and form. Most commonly they were oval or elongated and contained two or three pyknotic nuclei. The cell boundaries were not always very clear so that some of the cells seemed to form a syncytium. The cells correspond to those described as "leprosy cells" by Virchow. They were embedded in a connective tissue stroma, which originated from the "protective wall." The stroma increases in later stages, compresses the leprosy cells and finally replaces them, forming scar tissue. In other portions of the skin, lymphocytes, plasma cells, epithelioid cells and fibroblasts were noted. The changes that were found throughout the skin over the entire body gave the impression of a "wrap of leprosy." Hansen's bacillus was found throughout the skin lesions. These bacilli were mainly within the leprosy cells but were also present between them, sometimes in large clumps. Also degenerative forms of the leprosy bacilli were described which stained blue if stained according to Ziehl. The lymph nodes, but especially the cervical nodes, showed an almost complete disappearance of the lymphadenoid tissue. There were many leprosy cells with shrunken nuclei and a vacuolar cytoplasm. Some fields revealed giant cells with nuclei in the centers of the cells. The leprosy cells, according to the author, are derived from the reticulo-endothelial system. In this case the changes in the various organs are described in detail. The heart and kidneys showed no specific lesions, while in the liver, spleen and peripheral nerves



changes were marked. The author believes that a chronic hyperplasia of the reticulo-endothelial system, called for by the continuous phagocytosis of leprosy bacilli, is the essential finding in leprosy.

O. SAPHIR.

TWO TERATOMAS OF THE MEDIASTINUM. I. GORDON, Frankfurt. *Ztschr. f. Path.* **40**:224, 1930.

The author describes two teratomas of the mediastinum, which were clinically silent, but which were discovered at autopsy. One teratoma was found in a woman of 26. It showed many cysts, pancreatic tissue, with many large islands of Langerhans, thymic tissue with Hassall's corpuscles and a tissue that suggested gastric mucosa. Besides, fat tissue, connective tissue, smooth muscle fibers, bone and cartilage were also found as constituents of the tumor. Some portions contained cerebral structures. Many veins and arteries were found, some of which showed arteriosclerotic changes. The second tumor was found in a woman of 65. This tumor contained thyroid tissue which showed changes corresponding to those found in colloid goiter. Also, ovarian structures were present in the teratoma.

O. SAPHIR.

INTRATHORACIC CYSTS. B. ENTZ and D. OROSZ, Frankfurt. *Ztschr. f. Path.* **40**:229, 1930.

In an infant, aged 11 months, a cyst was discovered in the thoracic cavity by the x-rays. The cyst was removed, but the child died eighteen days after the operation. The cyst occupied the larger part of the right thoracic cavity, extending from the fourth rib to the diaphragm. It was covered by a smooth and glistening pleura and was adherent to the thoracic wall and to the mediastinum. The adhesions were separated with ease. The cyst was not attached to the esophagus, trachea or thoracic duct. It could easily be removed from the underlying diaphragm. There was no connection between the cyst and any of the abdominal organs. The cyst measured 2 by 7 by 10 cm. The thickness of its wall varied to 6 mm. The content of the cyst, when first obtained by thoracentesis, was a clear yellow and somewhat mucinous, sterile liquid. A few leukocytes, lymphocytes, red blood corpuscles and degenerated epithelial cells were found in the sediment. Neither succinic acid nor scolices could be detected. The content of the cyst subsequently became turbid and, later, purulent. Histologically, the wall of the cyst revealed structures that were identical with gastric mucosa and muscularis. The authors believe that the cyst arose from an heterotopic island of gastric mucosa in the esophagus. After obliteration of the ducts of glands in such an heterotopic island, cysts may be formed which later lose their connection with the esophagus. According to Lauche, such cysts should be classified as choristoma.

THE ORIGIN OF ENDOMETRIOMA: AN EXPERIMENTAL STUDY WITH CASE REPORTS. K. WOLFF, Frankfurt. *Ztschr. f. Path.* **40**:247, 1930.

This work was instigated by the finding of an endometrioma in a lymph node close to an endometrioma in the region of the round ligament. The lymph node was entirely separated from the tumor. The author believes that this finding tends to support Halban's theory, which explains endometriomas on the basis of a lymphogenous metastasis of parts of the uterine mucosa. Experiments with guinea-pigs in which an emulsion of uterine mucosa was injected into the inguinal region for the purpose of producing endometrioma in the inguinal nodes yielded negative results. Uterine tissue (mucosa and muscularis), if transplanted into the subcutaneous tissue of a guinea-pig, did not undergo necrosis. Injured uterine mucosa regenerated after it was transplanted. The author also tries to show that scars might originate in transplants. Transplants in castrated animals either remained alive and actually grew, as do those in noncastrated animals, or became atrophic and were replaced by scar tissue. The author reports two cases. One case was

that of a ruptured endometrioma of the ovary, simulating the clinical picture of ruptured tubal pregnancy. In the other case, there was a large endometrioma of the ovary in a woman, which gave the clinical picture of pernicious anemia. The opinion is expressed that endometriomas in scars following laparotomies are either implantations of uterine epithelium or manifestations of lymphogenous metastases of uterine mucosa.

O. SAPHIR.

PROLIFERATION OF VASCULAR ENDOTHELIUM. M. SILBERBERG, *Verhandl. d. deutsch. path. Gesellsch.* **25**:144, 1930.

By culturing the small and large vessels of the pia mater and chorioid plexus in heparinized blood plasma by the method of Maximow, the endothelium of the vessels was found to proliferate and form spindle-shaped cells that did not phagocytose and could not be differentiated from fibroblasts. The endothelium was differentiated from the histiocytes by vitally staining with carmine. The latter took up the dye while the former did not. The endothelium did not form blood cells. A transition of the histiocytes to fibroblasts could not be seen.

SOL ROY ROSENTHAL.

EXPERIMENTAL STUDIES OF THE GLIA CELLS. G. ROUSSY and C. OBERLING, *Verhandl. d. deutsch. path. Gesellsch.* **25**:162, 1930.

The experimental production of acute and chronic lesions of the brain in animals gave the following glial reactions: In acute lesions with necrosis, the astrocytes (macroglia) were destroyed and showed no reaction while the Hortega cells (microglia) proliferated. In chronic lesions, the astrocytes lost their prolongations and became rounded with vacuolated cytoplasm that contained fat. This change was similar to the changes in the microglia, but was not extensive. Following the intravenous injection of trypan blue alone, the glia cells do not take up the dye, but when potassium chloride is injected, these cells take up the dye, the microglia cells containing the greater amount. There is a great similarity in the phagocytic properties of the Hortega cells and astrocytes; these properties do not qualify a cell for the reticulo-endothelial group.

SOL ROY ROSENTHAL.

THROMBO-ANGIITIS OBLITERANS. H. DÜRCK, *Verhandl. d. deutsch. path. Gesellsch.* **25**:272, 1930.

In studying thrombo-angiitis obliterans, the author found that the primary lesions were in the small distal vessels. There is a subendothelial proliferation of fibroblasts and histiocytes which causes a thickening of the intima and a narrowing or complete obliteration of the lumen. The internal elastic membrane is tortuous but intact. The muscularis is also intact except for occasional leukocytic infiltration. As the process involves both arteries and veins, the term "endangiitis" would be more suitable.

Thombosis is secondary and not one of the primary factors as described by Buerger. That the condition is not an angiitis or phlebitis is shown by the intact internal elastic membrane and muscularis; in inflammatory conditions these structures are interrupted.

The possibility is strong that these lesions are similar to those in endocarditis lenta, in which subendothelial proliferation of the histiocytes and fibroblasts occurs in toxic infectious states.

SOL ROY ROSENTHAL.

INTESTINAL OBSTRUCTION FROM ENDOMETRIAL PROLIFERATION IN THE INTESTINAL WALL. BRUNO SCHULER, *Zentralbl. f. Chir.* **58**:399, 1931.

This report concerns a woman, aged 48, in whom intestinal obstruction was caused by a hard swelling, 4 cm. long, on the anterior wall of the junction of the

sigmoid with the rectum. This swelling proved to be due to endometrial proliferation, with stenosis. Symptoms traceable to the endometrial growth in the intestinal wall began in 1926; at times there were bloody stools during the menstrual period.

### Pathologic Chemistry and Physics

THE HYDROGEN-ION CONCENTRATION AND ACID-BASE EQUILIBRIUM IN NORMAL PREGNANCY. D. M. KYDD, *J. Biol. Chem.* **91**:63, 1931.

The hydrogen ion concentration of the blood in uncomplicated pregnancy is within the normal range. The alveolar carbon dioxide content is lowered, as is the carbon dioxide combining power of the blood. Less protein is present, the serum albumin fraction being decreased and the globulin fraction either unchanged or slightly increased. The concentrations of chloride, phosphate and undetermined acid ions in the serum are normal. The reduction in protein concentration appears to be balanced by a reduction in total base.

E. R. MAIN.

THE ALKALINITY AND THE PHOSPHATE CONTENT OF THE MORNING URINE. R. S. HUBBARD, S. A. MUNFORD, J. T. TYNER and C. B. ALLISON, *J. Biol. Chem.* **92**:xxix, 1931.

Persons with achlorhydria do not show the temporary increase in urinary alkalinity that follows the ingestion of food by normal persons.

ARTHUR LOCKE.

CHEMICAL CHANGES IN THE BLOOD OF THE DOG IN EXPERIMENTAL BILE PERITONITIS. A. M. ZIEGLER and T. G. ORR, *J. Exper. Med.* **53**:865, 1931.

Changes in the chemistry of the blood of dogs with experimental bile peritonitis are here reported. In all animals that died, bile ascites was found. Dogs dying of bile peritonitis showed a constant increase in nonprotein and urea nitrogen of the blood, and a fall in the chlorides.

AUTHORS' SUMMARY.

CALCIUM AND PHOSPHORUS IN THE BRAIN IN DIFFERENT CONDITIONS. ELIZABETH COWPER EAVES, *Brit. J. Exper. Path.* **12**:113, 1931.

An increase of calcium in the brain is relatively uncommon. It tends to occur in conditions in which there is gliosis without wasting of the brain. Increased calcium was found in the brains of two young patients in which numerous microscopic hemorrhages were present, and was not associated with the deposition of inorganic iron. In the brains in thirteen cases of degenerative nervous diseases (dementia paralytica, Huntington's chorea and arteriosclerotic insanity), in all of which there was wasting, there was increased calcium only in one and great diminution in phosphorus compounds in eleven. In the brains in four cases of Huntington's chorea, the calcium was diminished. In chronic epidemic encephalitis, increase of calcium in the brain is more common than in other conditions. In eight of nine cases, the phosphorus percentage was within normal limits. In epilepsy in six patients with status epilepticus, the calcium was within normal limits except in one patient with tuberous sclerosis. In three of the cases, the phosphorus was within normal limits. In the other three, in which epilepsy was associated with mental deficiency and dyspituitarism, the phosphorus was very low. This was probably due to disordered phosphorus metabolism in endocrine disorders rather than necessarily a result of status epilepticus. In one case of cretinism, the calcium was high and the phosphorus very low. In one case of Paget's disease, the amount of calcium was high. A low phosphorus content may exist with good myelinization, but poor myelinization is always accompanied by a decreased amount of phosphorus. It is concluded that nerve cells may be pathologically deficient in compounds of phosphorus, probably of a lipid nature. Abnormal function of the nervous system may be associated with chemical alterations in the brain, without necessarily any very definite histologic changes.

AUTHOR'S SUMMARY.

THE BLOOD GLUTATHIONE IN DISEASE. ROBERT PLATT, Brit. J. Exper. Path. **12**:139, 1931.

Observations on the glutathione content of human blood, as determined by iodine titration, show that the normal amount of glutathione present is about 30 to 50 mg. per hundred cubic centimeters, and that this is present almost exclusively in the corpuscles. In the majority of pathologic conditions, including cystinuria, the glutathione of the blood remains within normal limits, though cases of disease of the liver show rather wide variations. In anemia and some cyanotic states, the ratio of glutathione to corpuscles is increased. Studies of myelogenous and lymphatic leukemia show that glutathione is a constituent of both types of white corpuscles. A large amount of glutathione is also contained in pus cells.

AUTHOR'S SUMMARY.

A COMPARISON OF THE VITAL STAINING OF THE PARENCHYMA CELLS OF THE LIVER WITH ACID AND BASIC DYES. R. J. LUDFORD, Proc. Roy. Soc., London s.B. **108**:270, 1931.

Both acid and basic dyes form colored droplets in the parenchymal cells of the liver. The droplets are new cytoplasmic formations. They collect around the margins of the cells, bordering the intercellular bile capillaries. The degree of segregation depends on the state of functional activity of the cells. The capacity of a dye to induce the formation of droplets depends on physicochemical properties, its rate of diffusion and the extent to which it forms colloidal aggregates, rather than on acidic or basic properties.

E. R. MAIN

THE BROWN PIGMENT OF HEMACHROMATOSIS. MARTIN JACOBY, Biochem. Ztschr. **230**:225, 1931.

Partially purified preparations of the brown pigment that is deposited in the liver in hemachromatosis contain iron and nitrogen in the ratio of 66:94. The pigment has an absorption spectrum which resembles that of methemoglobin.

E. R. MAIN.

THE MAGNETIC SUSCEPTIBILITY OF BODY FLUIDS. F. K. T. SCHWARZ, Ztschr. f. d. ges. exper. Med. **76**:99, 1931.

Methods for the determination of the magnetic susceptibility of body fluids are discussed. In animals, it was found that changes in magnetic susceptibility of body fluids varied with changes in the hemoglobin content. It was found that some of the effects of certain iron compounds in anemia were caused by changes in magnetic susceptibility.

PEARL ZEEK.

STUDIES IN LIPOID METABOLISM. IGOR REMESOW and N. TAVASTSTYERNA, Ztschr. f. d. ges. exper. Med. **76**:419, 1931.

In both carnivora and herbivora cholesterol is by no means an indifferent substance. When given in colloidal form to dogs, either by way of the enteric tract or otherwise, hypercholesteremia is produced. The organism thereby protects itself against oversaturation with lipoids. To the lungs is attributed the specific function of "catching cholesterides" brought to them, either directly or through the blood stream. Not only do the lungs perform this mechanical function, but they also are the site of elaborate chemical processes concerned with esterization and transformation of cholesterol. The cholesterol-cholesterolester ratio in the blood functions as a buffer system to protect the organism against oversaturation with free cholesterol.

Lecithin appears to have very little toxic action in the body. When given in colloidal form to both herbivora and carnivora in suitable doses, a lecithinemia is produced. In herbivora a pronounced hypercholesteremia occurs also, but is absent or insignificant in carnivora.

PEARL ZEEK.

EXPERIMENTAL STUDIES IN FAT METABOLISM. IGOR REMESOW, D. MATTROSOWITCH and O. SAPALOWA, *Ztschr. f. d. ges. exper. Med.* **77**:67 and 100, 1931.

It was found that while adrenalin, nicotine and cholesterol lead to glycogenolysis in the liver, cholesterol plus insulin results in the storage of glycogen in the liver. It is concluded that insulin aids in the transformation of cholesterol into carbohydrate through an intermediate product "glychocholesteride," which has the characteristics of a physicochemical compound.

SANDER COHEN.

COLOR REACTIONS OF NUCLEIC ACID. P. THOMAS, *Ztschr. f. physiol. Chem.* **129**:10, 1931.

Several color reactions of saccharides of nucleic acids are described. If a small amount of a pentose solution or of its derivative is poured on the surface of a 0.3 per cent solution of beta-naphthol in pure sulphuric acid, an ultramarine blue ring appears. Pentoses of this kind are in yeast nucleic acid (arabinose, xylose, ribose). Thymonucleic acid gives a brownish-red ring similar to that of levulinic acid. Yeast nucleic acid or arabinose added to 1 per cent tryptophan in 50 per cent hydrochloric acid gives a pale green color. Thymonucleic acid gives a fuchsin red color which differs from that produced by common sugars, as the color is not taken up by chloroform or by ether and only partially by amyl alcohol. Beta-naphthol gives in sulphuric acid a pale yellow color. Apiose gives with this reagent a green color. Diluted glycuronic acid produces a blue-green ring, while concentrated glycuronic acid causes a brown ring with green edges. If a red ring appears with this substance, this is due to contamination probably with mentholglycuronic acid. The tryptophan reagent is prepared as follows: To an aqueous 2 per cent solution of tryptophan add an equal volume of concentrated pure hydrochloric acid. If kept in the dark, this reagent is good for two months. Add a few milligrams of the substance to be examined to 2 cc. of this reagent and heat for five minutes on a boiling water bath. Ordinary pentoses will then produce a pale green color. If more than from 1 to 2 mg. of sugar is added, the color changes into a light brown. This change is rapid if xylose is used. Apiose gives an orange-yellow color and levulin a pale pink one; hexoses give different colors (galactose and mannose, yellow to light brown; dextrose, pinkish purple with a bluish tint similar to thymonucleic acid; fructose, light red changing into dark reddish brown after an additional five minutes on the water bath).

WILHELM C. HUEPER.

IODINE IN BLOOD IN EXOPHTHALMIC GOITER. C. BULMAN, *Hospitaltid.* **74**:395, 1931.

In normal persons, the iodine in the blood varied from 0.008 to 0.018 mg. per hundred cubic centimeters. In conditions of thyrotoxicoses, there was an increase to from 0.01 to 0.154 mg. per hundred cubic centimeters. This increase of iodine in the blood may prove to have diagnostic value.

### Microbiology and Parasitology

TUBERCULOSIS IN THE MESENTERIC LYMPH NODES OF CHILDREN. MARION LEONARD, *Am. J. Dis. Child.* **41**:513, 1931.

The high incidence of tuberculosis in the mesenteric lymph nodes of children is brought out in this study. Contrary to prevailing opinion, there seems to be little relation between chronological age and calcification as a pathologic process in children. The significant value of 0.94 for the coefficient of association of tuberculous involvement of the mesenteric nodes and other tissues suggests that the intestinal lymphatic system plays an important rôle in tuberculous infection in

childhood. The fact that the mesenteric nodes are the only tissues in the body that are the sole demonstrable site of tuberculous infection in any single case (eighteen instances, or over one third of the cases studied) also points to the importance of the intestinal lymphatics in the infection of children with tubercle bacilli.

AUTHOR'S SUMMARY.

ACUTE GENERALIZED TUBERCULOSIS WITHOUT TYPICAL TUBERCLES. WILLIAM A. REILLY and ZERA E. BOLIN, *Am. J. Dis. Child.* **41**:582, 1931.

The case of an infant is reported, in which distribution of tubercle bacilli by the blood stream was followed by lesions showing few mononuclear cells, no giant cells, few lymphocytes and large numbers of polymorphonuclear cells, with great numbers of tubercle bacilli in each lesion. The explanation offered for this type of lesion is the large number of bacilli present, coupled with the nonresistance and lack of allergy of the patient.

AUTHORS' SUMMARY.

MULTIPLE PULMONARY ABSCESSSES SIMULATING TUBERCULOSIS. H. C. SWEANY, ASYA STADNICHENKO and KARL J. HENRICHSEN, *Arch. Int. Med.* **47**:565, 1931.

The clinical course and the pathologic and bacteriologic observations of a fatal chronic infection with Friedländer's bacillus are described. The micro-organism differed from that ordinarily described, in that it grew as well or better anaerobically than aerobically; it grew poorly on potato medium; it fermented lactose with acid and gas, and it produced coagulation in milk. The pathologic condition varied from a subacute to a chronic bronchopneumonia with progressive necrosis and formation of abscesses exhibiting a varied type of cellular reaction that varied from lymphocytes, monocytes and plasma cells to focal collections of polymorphonuclears. Fibroblasts and connective tissue appeared about and within the older lesions, and the walls of the cavities became lined with metaplastic cuboidal or squamous epithelium. An important feature of the disease was the continuous succession of lesions, usually from the apex to the base of the lung, each of which passed through the same evolution and changes from the acute (described by Moisejew, Kokawa and others) to the chronic form already outlined. The clinical aspects closely simulated chronic pulmonary tuberculosis, differing from it only in the general appearance of the patient, the irregularity in temperature and the obscure physical observations. The roentgen observations resemble those noted in chronic pulmonary tuberculosis.

AUTHORS' SUMMARY.

STREPTOCOCCIC SEPTICEMIA WITH VASCULAR LESIONS. J. M. STRANG and K. SEMSROTH, *Arch. Int. Med.* **47**:583, 1931.

An unusual case of sepsis due to *Streptococcus viridans*, with chronic and acute pancarditis and chronic and subacute nephritis, is described. A characteristic lesion that was found in the arterioles of both the heart and the kidneys is described at length. This lesion is regarded as causing the secondary degeneration of both the heart and the kidneys. The possible relationship of the anatomic lesions and the clinical course in a case of this type to low virulence of the invading organism and the absence of acute reaction on the part of the host is discussed and contrasted with more fulminating processes.

AUTHORS' SUMMARY.

BRUCELLA ABORTUS FROM A HUMAN FETUS. C. M. CARPENTER and R. BOAK, *J. A. M. A.* **96**:1212, 1931.

Bacteriologic examination of twenty-eight human fetuses and thirty-four placentas for *Brucella abortus* gave no evidence of infection except in one case. In this case typical cultures of *B. abortus* were obtained from the fetal viscera. Tests for agglutination and absorption of agglutinins confirmed the earlier results.

A guinea-pig inoculated, and eighty days later examined post mortem showed typical pathologic changes, while cultures of *B. abortus* were isolated from the liver and the spleen. The blood serum of this animal had a titer of 1:1,215 when tested against an abortus antigen.

EDNA DELVES.

EFFECT OF TOXINS AND VENOMS ON PROTOZOA. C. H. PHILPOTT, J. Exper. Zool. 56:167, 1930.

Under the conditions of the investigation, botulinus toxin, tetanus toxin, and ricin had no effect on *Paramecium caudatum*. The venom of eight snakes was found, however, to be highly toxic for paramecia. From a study of the effect of the venom of *Crotalus atrox* on eleven species of protozoa it appears that some species are more susceptible to the fatal action of the venom than others. The agent in this venom that is lethal to protozoa is thermolabile and is neutralized by crotalic antivenin.

Paramecia have been used as test animals in titrating the potency of venoms. Repeated titrations in which paramecia were used as test animals have shown small variation in the results for each venom. In these titrations the minimal lethal dose is defined as the smallest amount of venom per cubic centimeter of medium per animal required to kill paramecia within twenty-four hours. Paramecia have also been used in titrating the potency of crotalic antivenin, but the titer thus obtained does not correspond to that obtained when warm-blooded animals are used as test animals.

The agent that is responsible for the lethal action of venoms on paramecia is not the same in all venoms. In crotalic venoms the agent responsible for the death of paramecia seems to correspond to hemorrhagin, while in cobra venom it seems to correspond to neurotoxin, according to Noguchi's classification of the toxic constituents of venoms.

CHARLES H. PHILPOTT.

TISSUE SPECIFICITY IN ANTHRAX INFECTION. V. BURKE and L. A. BARNES, J. Immunol. 20:173, 1931.

The gelatinous infiltration in the subcutaneous tissue characteristic of anthrax infection occurs where the organisms are deposited, regardless of the location of the needle puncture through the skin. The pathogenicity of *Bacillus anthracis* is not specific for the skin.

AUTHORS' SUMMARY.

BACTERIOPHAGE AND ENFORCED DISSOCIATION. P. HADLEY and B. JIMÉNEZ, J. Infect. Dis. 48:176, 1931.

A pure S culture of *Bacillus paratyphosus* A was dissociated in 10 per cent homologous serum broth. Six transfers at forty-eight hour intervals gave R colonies. The rough colony was grown in 30 cc. amounts of broth over night, filtered through a Berkefeld N filter and tested against the S type for bacteriophage. The third filtrate yielded a phage. To overcome the objection that the blood of the rabbit immunized against the S type contained bacteriophage, a second experiment was tried. An S culture was grown in 30 cc. of 7.8 beef infusion broth for twenty-four hours at 37 C. and then was allowed to stand at the temperature of the room for four and one-half months. At this time plating showed only R colonies. With this culture the first filtrate revealed numerous lytic areas and a strong bacteriophage was built up. To test for the presence of phage in the original old culture, 1 cc. of the old culture was added to 30 cc. of broth and filtered. No lysis was observable on the plates. The view that bacteriophage is merely one aspect of the larger problem of microbic dissociation is discussed.

EDNA DELVES.

LYSOZYME IN THE DEVELOPMENT OF THE INTESTINAL FLORA OF THE NEW-BORN INFANT. L. ROSENTHAL and H. LIEBERMAN, J. Infect. Dis. **48**:226, 1931.

The rapid disappearance of the initial adventitious flora from the stools of nurslings is due to the action of lysozyme. The following facts sustain this hypothesis: Bacteria of the air are especially sensitive to the action of lysozyme. Human milk in contradistinction to cow's milk contains lysozyme. Lysozyme introduced with food passes through the intestinal canal and appears in the stool. The period of rapid destruction of air-borne bacteria in the intestines of infants coincides with the period in which lysozyme begins to appear in detectable quantity in the stools. Lysozyme is not found in the stools of infants fed cow's milk, who do not get it in their food supply, and rapid destruction of air-borne invaders does not occur in these infants. We may also assume that lysozyme, which persists during the entire period of breast feeding, continues to protect the intestines from invasion by bacteria of the air. Also, lysozyme inhibits the growth of *Bacillus coli* and apparently does not affect *B. bifidus*. Lysozyme may play a rôle in the stabilization of the intestinal flora of nurslings by eliminating *B. coli*.

FROM AUTHOR'S SUMMARY.

EGG YOLK AGAR MEDIUM IN TUBERCULOSIS. R. D. HERROLD, J. Infect. Dis. **48**:236, 1931.

The use of egg yolk in nutrient agar simplifies the cultivation of *Bacillus tuberculosis*, as well as of other bacteria that ordinarily require highly nutrient mediums. It can be used for both plates and slants. Comparative tests indicate that this method is much more certain than smears and not only as delicate as, but also much more prompt than, the inoculation of guinea-pigs. Initial cultures from a variety of infected exudates demonstrate that the value of cultures in this medium in clinical diagnosis is greater than that of smears. A moist atmosphere and a temperature ranging between 34 and 36 C. are essential for optimum growth of tubercle bacilli on the egg yolk medium. The yolk of one egg is added to 150 cc. of melted agar ( $p_H$  7.5) at a temperature of 60 C. The agar is allowed to cool to about 40 C., and about 25 cc. poured into each Petri dish.

AUTHOR'S SUMMARY.

BACTERIAL FLORA OF RESPIRATORY SYSTEM OF NORMAL DOGS. H. LIVINGSTONE and W. E. ADAMS, J. Infect. Dis. **48**:282, 1931.

One third of the dogs that appeared normal on external examination presented gross evidence of bacterial contamination of the tracheobronchial tree at autopsy. All the dogs were found by bacteriologic examination to have contamination in the parenchyma of the lung. Occasional contamination of the tracheobronchial tree of normal dogs was determined by bacteriologic examination. Excessive contamination of this area was demonstrated in dogs presenting gross contamination. Bacteria were present in the blood in four of eleven normal dogs. Contamination of the blood stream was found more frequently in dogs showing gross contamination of the tracheobronchial tree.

AUTHORS' SUMMARY.

ACIDITY IN BRUCELLA CULTURES. S. H. McNUTT and P. PURWIN, J. Infect. Dis. **48**:292, 1931.

Broth and peptone solution in which *Brucella* was grown without sugar became strongly alkaline. When dextrose, levulose, galactose, xylose or arabinose was added, the medium still became alkaline, even though determinations of sugar showed a loss. When sugars were added to a solution of nutrose and serum, and the medium was inoculated with *Brucella*, very evident traces of acid were often produced in dextrose, levulose, galactose and xylose. With arabinose, greater quantities of acid were formed. When *Brucella* was limited to a sugar as the



only source of carbon, experiments indicated that growth took place in both arabinose and xylose. It was impossible to classify the strains according to source when grown in carbohydrate mediums.

AUTHORS' SUMMARY.

BACTERIAL FLORA OF INTESTINAL SEGMENTS. VERA SMITH, *J. Infect. Dis.* **48**:295, 1931.

The organisms most commonly found in isolated loops of the small intestine belong to the *Bacillus coli*, *Streptococcus*, *Staphylococcus* and *Clostridium welchii* groups. *Cl. welchii* is present in the loop in a large percentage of dogs soon after operation, but tends to disappear from the isolated segment after a few weeks. No apparent toxemia is produced, although *Cl. welchii* may be present in the loop for some time. Isolated intestinal loops do not tend to become sterile. Contents of segments from different levels do not differ more than do those of segments of the same level. The same types of organisms are present over a long range of the  $p_H$  scale. The number of organisms per cubic centimeter of loop material varies greatly from day to day.

AUTHOR'S SUMMARY.

RELATION OF STREPTOCOCCI TO THE FILTRABLE VIRUS OF EPIZOOTIC ENCEPHALITIS OF THE FOX. E. C. ROSENOW, *J. Infect. Dis.* **48**:304, 1931.

A streptococcus with distinctive cultural, serologic and neurotropic properties has been isolated consistently from the brains of young foxes and dogs that have succumbed to encephalitis following the inoculation of virus. The characteristic symptoms and lesions produced by the virus were closely simulated following the injection of gaged doses of a strain of the streptococcus after it had passed through from few to many rapidly repeated subcultures. From one strain of the streptococcus, which was isolated from the brain of a dog that succumbed to the virus and which was carried through thirty-three subcultures, a filtrable virus seemingly has been produced. The period of incubation, the symptoms during life and the lesions after death in animals given injections of the artificial virus were the same as for the natural virus. The streptococcus was isolated from the brain with the same difficulty as was the natural virus. The cultural characteristics, the serologic reactions and the virulence of the streptococcus from the natural virus and the artificial streptococcus were the same. The streptococcus from the natural virus protected animals against the artificial virus. The artificially produced virus resisted glycerination for from fifteen days to six months. The method of injection and that of preparing the type of antigen used for immunization are relatively simple and practical in application for prophylactic immunization against the highly fatal disease in foxes. The conclusion that the streptococcus isolated has etiologic significance in the epizootic encephalitis of foxes, and that it gives rise to the filtrable virus seems warranted.

FROM AUTHOR'S SUMMARY.

SPLENIC LESIONS IN CANINE RABIES. R. D'AUNOY and J. L. BEVEN, *J. Infect. Dis.* **48**:335, 1931.

Gross and microscopic study of the spleen in 100 rabid and 50 nonrabid dogs failed to establish any marked relative splenomegaly or microscopic splenic changes that could be considered pathognomonic for canine rabies.

AUTHORS' SUMMARY.

EXPERIMENTAL SCARLET FEVER. T. TOYODA, Y. FUTAGI and M. OKAMOTO, *J. Infect. Dis.* **48**:350, 1931.

Experimental scarlet fever was produced in three children inoculated with freshly isolated strains of scarlet fever streptococci. Five children treated with old strains yielded no case of scarlet fever. Filtrates of shaken streptococcus cultures did not cause the disease in susceptible children, strengthening the evidence

that scarlet fever is caused by the scarlatinal hemolytic streptococcus and not by a filtrable body attached to the streptococcus.

EDNA DELVES.

BACTERIOLOGIC EXAMINATION OF MILK. F. BOERNER and M. O. ROBINSON, J. Infect. Dis. **48**:372, 1931.

Spreaders on agar plates used in counting milk was not found to be due to faulty sterilization or to faulty technic. The condition of the surface and the depth of the agar influence the spreading of colonies of *Bacillus subtilis* and *B. proteus*. Plates dried over night and containing only 10 cc. of agar per plate prevent the spreading of surface colonies.

EDNA DELVES.

BRUCELLA BACTERIOPHAGE. R. GWATKIN, J. Infect. Dis. **48**:404, 1931.

Five samples of feces from infected and four from uninfected cows were examined for the presence of *Brucella abortus* phage. Nine samples of milk from infected and four from uninfected animals were also examined. One infected fetus, uninfected twin fetuses, six apparently normal fetal membranes and three samples of blood from infected cattle were tested. There was no evidence of bacteriophage in the material examined. Poor growth or absence of growth on agar was shown to be due to water of condensation. No lytic agent could be demonstrated in the clear or in the partially inhibited areas. Attempts to lyse *B. abortus* with *Salmonella pullorum* phage were unsuccessful.

FROM AUTHOR'S SUMMARY.

BACTERIAL GROWTH IN UDDERS OF COWS. H. R. CURRAN, J. Infect. Dis. **48**:408, 1931.

Bacteria in the udders of living cows remain numerically constant, but increase rapidly immediately following death and the removal of the blood. Rapidly growing invasive types of streptococci are most affected. In some cows the flora do not increase during the postmortem period of incubation; slow-growing micrococci and streptococci usually comprise this group. The increased multiplication frequently attending the withdrawal of blood suggests that the circulatory system is directly or indirectly associated with the formation of the bactericidal substance in milk.

FROM AUTHOR'S SUMMARY.

THE VIRUS OF PSITTACOSIS. S. P. BEDSON and G. T. WESTERN, Brit. J. Exper. Path. **11**:502, 1930.

The guinea-pig is susceptible to the virus of psittacosis, and this animal can be used for maintaining strains, or what is probably more important, for their titration. Quantitative filter and centrifuge experiments indicate that the virus of psittacosis is relatively large. The relation between the virus and the micro-organismlike bodies present in virulent material is discussed. Virulent mouse spleen loses its activity rapidly when stored in 50 per cent glycerol, but much less rapidly when placed in fiftieth-molar phosphate,  $p_H$  7.6. A phosphate suspension of mouse spleen stored at 6 C. maintained the virulence unaltered for thirty-four days. Beyond that time the virulence appeared to fall off rapidly. Freezing would seem to be a satisfactory method of conserving the virus in normal spleen, at any rate for periods up to two months. Evidence is produced in support of the immunologic identity of strains of psittacosis virus of various origins.

AUTHORS' SUMMARY.

LATENT AND OCCULT TUBERCULOSIS OF BRONCHIAL GLANDS. W. M. CUMMING, S. J. HARTFALL and J. G. THOMSON, J. Path. & Bact. **34**:157, 1931.

The results of a bacteriologic and histologic investigation of bronchial glands in nontuberculous subjects is recorded. In 7 of 108 cases, tubercle bacilli of human

type were isolated. In 3 of these cases there were histologic evidences of tuberculous infection, while in 4 the glands were histologically normal. In no case bacteriologically negative was there a histologic lesion.

AUTHORS' SUMMARY.

EFFECT OF DIET IN EPIDEMIC INFECTIONS IN MICE. W. W. C. TOPLEY, M. GREENWOOD and J. WILSON, *J. Path. & Bact.* **34**:163, 1931.

Mice fed on a basal diet of whole wheat flour, casein, butter and a salt mixture showed no advantage over mice fed on a diet of whole oats, milk and water, as regards their liability to contract a fatal infection during an experimental epidemic caused by *B. aertrycke*. The addition of an excess of fat, butter or lard, or of a vitamin A concentrate, to this diet appeared to react unfavorably on the mice at risk. This unfavorable action was not manifest when the mice were infected by intraperitoneal inoculation. Under these conditions there was a suggestion that the mice fed on the diet of whole oats, milk and water were slightly less resistant than the other groups; but the differences observed were insignificant. The addition of cabbage, carrots or mangolds to the normal diet did not lessen the severity of the epidemic which followed exposure to infection. The addition of carrots appeared to react unfavorably; the effect produced by the addition of mangolds was in the same direction, but the difference in this case was not significant. No evidence was obtained that any of the modifications in the customary diet so increased the resistance of the mice as to produce a significant decrease in mortality under epidemic conditions.

AUTHORS' SUMMARY.

CHANGES IN THE BLOOD SUGAR AND BLOOD PHOSPHORUS IN RABBITS FOLLOWING THE INJECTION OF SUSPENSIONS OF *BACT. AERTRYCKE*. M. E. DELA-FIELD, *J. Path. & Bact.* **34**:177, 1931.

Intravenous inoculations of rabbits with dead suspensions of *Bacterium aertrycke* and of the bacterial filtrate cause a hyperglycemia which is followed by a hypoglycemia. When death of the animal occurs, it is in the hypoglycemic phase. The inorganic blood phosphorus lessens in amount during hyperglycemia and increases again, often above the initial value, during the hypoglycemic phase. Organic acid-soluble blood phosphorus in many cases tends to increase during hyperglycemia and decreases during hypoglycemia. Treatment of the bacterial bodies by alcohol, acetone, steaming or autoclaving does not alter the type of the chemical response. The heated organisms are as active chemically as the unheated, although their enzymic activity as tested by the methylene blue technic is much reduced. Diphtherial toxin does not give the same type of response. There is no immediate change in the sugar and the phosphorus content of the blood, although these begin to rise after two hours and reach a very high level after two days.

AUTHOR'S SUMMARY.

ULTRAVIRUSES INDUCING NEUROTROPIC ECTODERMOSSES. C. LEVADITI, *Ann. Inst. Pasteur* **45**:673, 1930.

The infections considered are those induced by ultraviruses demonstrating an elective affinity for tissues derived from the ectoderm, as variola, vaccinia, epidemic encephalitis, experimental herpes, poliomyelitis, rabies, et al. The author concludes: "The neurotropic ectodermoses, belonging to a group of leukoneuraxitis, have a different pathogenesis according to which the ultravirus has an elective affinity for the microglia or for the oligodendroglia. In the first case, the leukoneuraxitis reveals inflammatory and lipolytic characteristics, whereas in the second it proceeds with a disturbance of the lipid metabolism lying in the oligodendroglia (lipotrophic leukoneuraxitis). However that may be, the result is a grouping of the evidence to the effect that there is demonstrated an elective affinity of neurotropic ultraviruses for such and such a group of cellular elements of the axone which

determines the clinical type, the evolution and the distinctive histopathologic characteristics of neurotropic ectodermoses." These conclusions are supported by twenty-two illustrations of gross and histologic specimens, which are briefly discussed.

M. S. MARSHALL.

### Immunology

VACCINIA: SUSCEPTIBILITY OF MICE AND IMMUNOLOGIC STUDIES. M. J. ROSENAU and H. B. ANDERVONT, *Am. J. Hyg.* **13**:728, 1931.

Our attempts to vaccinate mice on the skin of the abdomen seemed to fail. We could not believe, however, that this particular species among rodents could have a natural resistance in view of the wide host susceptibility to vaccine virus. Our first thought was that a successful "take" might be obtained by increasing the vascularity of the skin. This we did by various physical and chemical means, but without success. Next, we tried several methods of enhancing the virulence of the virus, but again failed to satisfy ourselves that a successful "take" had been produced. Attempts to alter the susceptibility of the mice were also negative. Active vaccine virus was found in scrapings from the skin six days after vaccination. We did not consider the presence of the live virus in the skin as conclusive evidence of a "take," because definite assurance of its multiplication was not available. Such an occurrence might simply mean survival. We know furthermore that vaccine virus is widely distributed throughout the body in vaccinia. The skin of the abdomen of mice is so thin that only occasionally and with special strains of vaccine virus will a reaction be produced that can be considered successful, and even then the "take" is so small and indefinite that in no case could we say with anything like finality that a primary vaccination had been produced. Nevertheless, when these mice were subsequently tested they were found to be immune to vaccinia, clearly indicating that infection had taken place even in those mice on which no visible evidence of skin reaction could be discerned. As further evidence that the thickness of the epidermal layer of the skin is the determining factor in a visible "take," we found that typical vesicles follow vaccination upon the pad of the foot or the skin of the tail. These observations confirm the pathologic picture in that the papular and vesicular lesions of a vaccine "take" reside mainly in the epithelium. In another series of tests it was demonstrated for the first time that mice are susceptible to vaccinia by inoculating the virus into the brain. This is true, however, only for an especially virulent strain rendered specifically active by particular technic. We carried the virus through a series of 28 mouse-brain passages extending over a period of six months and showed that vaccine virus was responsible for the symptoms observed by cross immunity tests with rabbit neuro-vaccine and calf dermovaccine. The symptoms are tremors, lethargy and sometimes a brief period of terminal excitement and convulsions. These results should not be interpreted as vaccinal encephalitis as usually understood. Furthermore, microscopic examination of the brains of mice after intracranial inoculation of vaccine virus failed to reveal lesions described as characteristic of vaccinal encephalitis.

AUTHORS' SUMMARY.

FURTHER STUDIES ON THE IMMUNOLOGY OF THE PNEUMOCOCCUS. W. A. JAMIESON and H. M. POWELL, *Am. J. Hyg.* **13**:823, 1931.

Under conditions not entirely understood some pneumococci produce toxic substances not greatly unlike those that have been described in studies of various streptococci. These can be detected and measured by human skin tests and similar tests carried out upon certain breeds of rabbits. Specific skin test neutralizing pneumococcus serums have been produced by subcutaneous injection of horses. The "skin test" potency of such serums appears to be concentrated to a moderate degree in the refining of globulins by well known salting out methods. These concentrated materials compare very favorably with the best

scarlet fever streptococcus serums on the basis of skin test estimations of potency. They contain only a small amount of animal protective body. A limited clinical use of serum of such nature indicates that further tests may well be carried out with combined antibacterial and anti skin toxic serum.

AUTHORS' SUMMARY.

HETEROPHILE ANTIGEN IN PNEUMOCOCCI. G. HOWARD BAILEY and MARY SHAW SHORB, *Am. J. Hyg.* **13**:831, 1931.

Rabbits injected with a large number of cultures of different types of pneumococci and one culture classified as a green-producing streptococcus, developed a potent anti-sheep hemolysin in their serums. Such an antibody is also formed in rabbits infected with pneumococci or fed the killed cultures. The hemolysin in the serums of such rabbits gives the characteristic reactions of the heterophile antibody stimulated in these animals by the injection of boiled sheep red blood corpuscles. Antipneumococcus hemolytic antibody may be removed from serums by absorption either with homologous or heterologous boiled pneumococci or with boiled sheep corpuscles. Such an antibody has the property of primary toxicity for the guinea-pig. Rabbits immunized with sheep red blood corpuscles are relatively resistant to intravenous infection with type I pneumococcus. These results indicate that heterophile antigen and antibody have a definite biologic significance in pneumococcus infections.

AUTHORS' SUMMARY.

THEORETICAL CONSIDERATIONS IN CONNECTION WITH A COLD INCUBATION COMPLEMENT FIXATION TEST. B. S. LEVINE, *Am. J. Syph.* **15**:225, 1931.

The precipitation of the antigenic complex from the colloidal suspension in the serum is essential to the fixation of complement. Separation or conglomeration of the complexes as sensitized entities is firmer and more complete, though slower, at from 8 to 10 C. This is due to the fact that the phase augmentation and the concomitant sensitization of the antigen complex are reversible processes, more pronounced at 37.5 than at from 8 to 10 C. Since the antigen complex is not a specific immunochemical entity, it causes complement to be fixed in non-syphilitic serums as well. However, in the majority of syphilitic serums, the reaction curves, as a rule, extend farther along the abscissa, and their crests are higher than in the reaction curve of all other serums. Therefore, the general reaction can be conveniently divided into levels. The lower the reaction level, the greater is the number, and the more varied the type, of serums that will respond to the influence of the so-called antigenic reagent. By raising the level, a number of serums can be excluded. It would appear, theoretically, that a diligent search might disclose a reaction level at which only syphilitic serums would be involved. However, since the intensity of any serum reaction, that is, the path followed by its curve, is determined not only by the type of immuno-reacting substances in the serum, but by their concentration and by serum factors which influence dispersion as well, the selection of a level at which all syphilitic, and at the same time only syphilitic, serums could be made to react is unattainable with the present insufficient knowledge.

AUTHOR'S SUMMARY.

ANAPHYLACTIC AND TUBERCULIN TYPES OF HYPERSENSITIVENESS. L. DIENES, *J. Immunol.* **20**:333, 1931.

The crystalline egg albumin and egg globulin differ considerably in their relationship to the different manifestations of hypersensitiveness. The crystalline egg albumin tends to sensitize the organism to those manifestations of the hypersensitiveness which are mediated by antibodies. The egg globulin tends to sensitize to those in which the active immunization plays the more important rôle. The difference between the antigens lies in their capacity to produce a different type of sensitization. We find no marked difference between them concerning their capacity to produce antibodies or to elicit the reactions themselves. The

observations with crystalline egg albumin and egg globulins present an analogy to the behavior of certain bacterial antigens that we usually find connected either with the tuberculin or with the anaphylactic type of sensitiveness. We have given a short review of our experiences concerning the capacity of different antigens to produce the tuberculin type of skin sensitiveness. With all innocuous antigens that we tested—as eggwhite and animal serums—we succeeded in producing this sensitiveness. With bacterial antigens we have not so far succeeded in producing it.

AUTHOR'S SUMMARY.

LOSS OF DIPHTHERIA ANTITOXIN IN HUMAN URINE. J. M. NEILL, E. L. GASPARI and R. A. MOSLEY, *J. Immunol.* **20**:347, 1931.

The paper reported a study of the elimination of "natural" diphtheria antitoxin in human urine. The urines were divided into three classes: those containing no detectable serum protein as obtained from persons whose renal permeability to protein was normal; those containing moderate amounts of protein as obtained from persons (cardiac failure and orthostatic albuminuria) of abnormal renal permeability but with no lesions in the lower urinary tract; those obtained from persons with lesions in the lower urinary tract that might have afforded opportunity for whole serum to leak into the urine without filtration through the kidney. When derived from persons with normal kidney permeability, detectable amounts of antitoxin (0.002 to 0.004 unit per 1.0 cc.) were found only in the urines voided by a man possessing an exceptionally high (15.0 units) concentration in his blood stream. When the urines contained moderate amounts of serum protein, antitoxin was contained if the donor of the urine possessed only moderately high amounts in his blood. The occurrence of antitoxin in urine containing no detectable serum protein is discussed. Data on the daily output of antitoxin showed that elimination by way of the urine can account for only a small portion of the drop frequently observed in the antitoxin content of the blood. However, none of the pathologic urines examined contained more than moderate amounts of albumin, and it is suggested that in cases of acute nephrosis or other clinical conditions in which large amounts of serum protein are eliminated, urinary elimination of antitoxin might be sufficient to cause a significant loss in the immunity reserves of the body.

AUTHORS' SUMMARY.

SPONTANEOUS IMMUNIZATION OF RABBITS TO VACCINE VIRUS. F. DURAN-REYNALS, *J. Immunol.* **20**:389, 1931.

The examination of the results shown in table I leads to the conclusion that repeated injury from injection and bleeding increased the possibility of spontaneous infection in an infected room. That this possibility was not materially influenced by the injection of an enhancing factor is evident by the fact that some animals injected with spleen or tumor tissue without enhancing power became immune as well as those receiving testicle extract. The part played by repeated injury is indicated by the low incidence of spontaneous immunization (13 per cent) among the tumor animals which received a single injection, compared with the much higher figure (38 per cent) for the rabbits repeatedly injected with the testicle and spleen extracts. The easy possibility of unnoticed infections leading to development of subsequent immunity has an important bearing on the interpretation of the recently much discussed question of the production of immunity by killed vaccine virus.

AUTHOR'S SUMMARY.

TUBERCULOTOXINS. F. EBERSON and M. A. SWEENEY, *J. Immunol.* **20**:395, 1931.

Experiments are reported the results of which indicate that filtrates of cultures of tubercle bacilli in hormone broth contain substances that are toxic for tuberculous guinea-pigs. These toxic substances were neutralized by the serum of a goat immunized with these substances over a long period.

ACID PRECIPITATION OF DIPHTHERIA TOXIN. W. E. BUNNEY, J. CIANCARULO and M. KIAMIL, *J. Immunol.* **20**:417, 1931.

A study has been made of yields of diphtheria toxin obtained by acid precipitation of the crude toxin. A comparison has been made between the yields obtained when the acid precipitate is centrifugated immediately after acidification and when twenty-four hours' storage in the cold intervenes between the acidification and the centrifugation. Results are obtained which indicate that a satisfactory method for large scale purification of diphtheria toxin would consist in the addition of acid to the point of maximum precipitation followed by immediate centrifugation, washing and solution of the precipitate at the desired concentration.

AUTHORS' SUMMARY.

THE SPEED OF FLOCCULATION OF DIPHTHERIA TOXIN. W. E. BUNNEY and M. KIAMIL, *J. Immunol.* **20**:433, 1931.

A toxin that on acidification yields a slight precipitate flocculating slowly in the Ramon test can be made to yield a rapidly flocculating precipitate by the addition to the toxin of a protein foreign to the diphtheria bacillus but precipitating at the same  $p_H$  as the toxin itself.

AUTHORS' SUMMARY.

TRANSMISSION OF SENSITIZATION FROM MOTHER TO CHILD. S. D. BELL and Z. ERIKSSON, *J. Immunol.* **20**:447, 1931.

Five cases of asthma and five of hay-fever in pregnant women were studied by the Prausnitz-Küstner method as to the passage of antibodies through the placenta from mother to child. In each case the ability of the mother's serum, while she was carrying the child, to give a local passive transfer to a normal individual was established, in most cases with the serum diluted 1:40 and in one case as high as 1:320. In no case could similar properties be shown in the undiluted cord blood or the undiluted child's blood.

AUTHORS' SUMMARY.

DIPHTHERIA ANTITOXIN IN HUMAN SALIVA. J. Y. SUGG and J. M. NEILL, *J. Immunol.* **20**:463, 1931.

The concentration of antitoxin in the saliva was directly related to the concentration in the blood; the average ratio of  $\frac{\text{saliva antitoxin}}{\text{serum antitoxin}}$  was  $1.4 \cdot 10^{-3}$ ; the ratio remained of the same order of magnitude in spite of the wide range in serum antitoxin concentrations (1.25 to 60 units) occurring in the group of people studied. Exception to the usual ratio appeared to be characteristic of certain individual persons. The data indicate that during a year's time the antitoxin swallowed with the saliva would be equivalent to approximately 25 per cent of the total amount in the blood. Unless reabsorption occurs, the gastrointestinal tract must be considered an important channel of loss.

AUTHORS' SUMMARY.

INFUSION AND INFUSION-FREE DIPHTHERIA TOXIN. M. B. KIRKBRIDE, K. C. BERTHELSEN and R. F. CLARK, *J. Immunol.* **21**:1, 1931.

Diphtheria toxin of uniformly high potency has been produced in infusion-free peptone medium on a routine basis for a period of nearly two years. The infusion-free toxin compares in all respects very favorably with routine infusion toxin for the immunization of horses in the production of diphtheria antitoxin. This toxin has also been used extensively in comparative flocculation-reaction tests and found in all respects to compare favorably with the infusion toxin. The flocculation-reaction test for standardization of diphtheria antitoxin proved more accurate when relatively large volumes were measured by pipettes than when the micrometer-

syringe method was used. Closer agreement was found between the flocculation-reaction-test and the subcutaneous animal method for the determination of antitoxic content than between the flocculation and the intracutaneous animal test, or between the subcutaneous and the intracutaneous test. The flocculation-reaction test was satisfactory for preliminary estimations of the antitoxin content of the concentrated as well as of the untreated serum. Besides its other advantages, the great saving in production cost as compared with the usual meat-infusion toxin makes the infusion-free peptone toxin an important economic factor where large amounts of toxin are required for the production of diphtheria antitoxin, for standardization, and possibly also for the active immunization of persons with toxoid.

AUTHORS' SUMMARY.

SURFACE PHENOMENA IN THE REACTION OF TOXIN AND ANTITOXIN. K. C. BERTHELSEN, *J. Immunol.* **21**:21, 1931.

It has been demonstrated that the toxin-antitoxin flocculation first takes place at the liquid-air surface, where the surface energy is at a minimum. Tests of toxin made during its production in infusion-free peptone medium and particularly after purification by ultrafiltration, showed that the shorter the flocculation-reaction time the higher the surface tension, measured in dynes. The results of tests made with an antitoxic serum in which the protein particles had been denatured by heat, showed that there exists a parallelism between the flocculation-reaction time and the surface tension in dynes. These experiments indicate that changes in the colloidal state of the toxin-antitoxin interfaces probably result in changes in the reaction time.

AUTHOR'S SUMMARY.

FLOCCULATION-REACTION TIME IN THE COURSE OF IMMUNIZATION AND THE CHANGES OF THE PROTEINS. K. C. BERTHELSEN, *J. Immunol.* **21**:43, 1931.

The flocculation-reaction time in general shows a continuous increase during the immunization period. This reaction time is in no way directly related to the titer; but it does seem possible in many instances to find a relationship to the ability of the individual horse to produce antibodies. It has been demonstrated that the increase in flocculation-reaction time is not related to quantitative changes in protein fractions. It must, apparently, be due to qualitative differences. No qualitative differentiation in the protein complexes could, however, be demonstrated by means of surface-tension measurements. On the basis of this study it is, therefore, concluded that it is necessary to investigate further the electro-kinetic phenomenon in order to gain a better knowledge of the qualitative characteristics of the immune protein.

AUTHOR'S SUMMARY.

THE DISTRIBUTION OF ELECTROLYTES IN SERUM DURING IMMUNIZATION. K. C. BERTHELSEN and P. P. MURDICK, *J. Immunol.* **21**:69, 1931.

On the basis of the experimental data obtained in a study of serum from horses undergoing immunization with diphtheria toxin, the diffusible calcium in the serum displayed a noticeable tendency to decrease in the twenty-four-hour period following the injection of large amounts of toxin. A somewhat similar tendency was also noted in the case of the total calcium and the total base. There was considerable fluctuation in the amounts of total and diffusible phosphorus during the immunization period, but with no apparent relationship to the development of immunity, although these values tended to decrease as the antitoxic potency increased. It is suggested that this drop in diffusible calcium, and possibly also in other electrolytes, is a chemical factor related to the immunologic stimulation process.

AUTHORS' SUMMARY.



RÔLE OF THE NERVOUS SYSTEM AND OF CONDITIONAL REFLEXES IN IMMUNITY.  
S. METALNIKOV, *Ann. Inst. Pasteur* **46**:137, 1931.

By the use of methods developed by Pavlov, immunologic responses were obtained following the use of conditional stimuli. These were produced by following the injection of antigen in rabbits with various external stimuli (scratching or heating of the skin, ringing of a bell, etc.). After from twenty to twenty-five such injections followed by stimuli the animals were left alone for about fifteen days; then the stimulus alone without the injection of the antigen was followed in some animals by an immunologic response. The responses that were obtained included: (1) leukocytosis, following the injection of vibrio of cholera plus stimulus, and later following the stimulus alone, (2) agglutinins for the same micro-organism and (3) cellular reactions in the peritoneal cavity. Nonspecific immunologic responses were obtained by injecting guinea-pigs with filtrates of staphylococci or with *Bacillus anthracis*, accompanied by external stimuli. After an interval the external stimulus alone was repeated, followed by an injection of fatal doses of *Vibrio* of cholera. The animals so prepared survived while the controls died. The rôle of the central nervous system in immunity was studied in certain insects. The third thoracic ganglion seemed to be of importance for both natural and acquired immunity. The author's experiments with higher animals were not successful. However, the recently published work of Speransky is quoted (*The Nervous System in Pathology*, Leningrad, 1930, in Russian), in which all sensory and motor nerves were removed from the left ears of two rabbits. To both ears of one of them Besredka's antiviral was then applied, followed in twenty-four hours by an injection of a culture of staphylococci. The control animal and the left denervated ear of the experimental animal showed a severe inflammation, while the right ear was left intact.

I: DAVIDSOHN.

A SPECIFIC WASSERMANN REACTION IN HEPATIC DISTOMIASIS. G. CELLI,  
*Policlinico* **38**:171, 1931.

A girl, aged 11 years, suffering from hepatic distomiasis but without any signs of syphilis, gave a positive Wassermann reaction which disappeared as the distomiasis improved under treatment.

MILK ANTIBODIES IN THE SERUM OF NURSINGS. P. GYÖRGY, E. MORO and  
E. WITEBSKY, *Klin. Wchnschr.* **10**:821, 1931.

Complement binding antibodies for milk were present in the serums of 40 of 60 infants on a formula diet and normal nutritional conditions. These antibodies correspond in their properties to immune substances (precipitins) and differ from those significant in the genesis of eczema infantum.

AUTHORS' SUMMARY.

THE WASSERMANN REACTION WITH FLUIDS OF THE TISSUES. LEO VON  
WIKULLIL, *München. med. Wchnschr.* **78**:708, 1931.

The results of Wassermann tests with fluids from cantharides vesicles are comparable to those with serum.

AUTHOR'S SUMMARY.

SPLEEN AND PHAGOCYTOSIS. ERWIN SCHLIEPHAKE, *Ztschr. f. d. ges. exper. Med.* **77**:204, 1931.

An extract of spleen is described which appears to stimulate phagocytosis. Staphylococci and tubercle bacilli were used as test organisms. The typical effect takes place even with minute quantities of the splenic extract, but it is not proportional to the amount used. The extract is inactivated by boiling.

SANDER COHEN.

SPECIFIC ANTIGENS AND ANTIBODIES OF THE BRAIN. P. SKWIRSKY, B. ARONOWITSCH and N. NEJOLOWA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:195, 1931.

This is a check of the report of Georgi and Fischer on the demonstration in the blood serum of brain antibodies with the help of the absorption technic of Witelski. Lipoids of brain and heart with and without cholesterol are added to the serums for the purpose of absorbing the corresponding antibodies. Subsequently complement fixation tests are carried out with the same antigens. The results of Georgi and Fischer are refuted by pointing out the possible error in technic; even prolonged centrifugation does not remove the lipoids added to the serum. Brain antigen proved less satisfactory than heart antigen in complement fixation tests with serums and spinal fluids from patients with neurosyphilis.

I. DAVIDSOHN.

THE SPECIFICITY OF IMMUNE SERUM AND THE SERUM DIAGNOSIS OF BACTERIA. A. AOLI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:217, 1931.

The antigenic substances in bacteria can be divided into two groups of partial antigens, one of which is shared with many related species, the other being specific for the particular species. Both groups of partial antigens are represented in the immune serum by corresponding antibodies. In certain species the specific partial antigens are present in single individuals. By proper absorption specific immune serums can be produced, which permit the differentiation as well as the demonstration of interrelationships between the bacterial species. In this manner it was possible to produce specific agglutinating serums for the various bacteria of the *Salmonella* group.

I. DAVIDSOHN.

ABSORPTION EXPERIMENTS WITH HEMOLYTIC ANTIBODIES. TSIEN-YUNG TSÜ, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:223, 1931.

Contrary to previous conceptions, absorption of hemolytic antibodies by kaolin from undiluted serum was demonstrated. The absorbed hemolytic amboceptor was shown by its action on red blood cells, and it could be separated from the absorbent by proper means. Sodium hydroxide interfered with the absorption of the hemolytic antibody by kaolin, while hydrochloric acid was indifferent or even favorable. The already effected combination of kaolin and hemolytic antibody was dissolved by the action of diluted sodium hydroxide as well as of inactivated sheep or rabbit serum.

I. DAVIDSOHN.

ADVACCINE (ANTIDENATURED VACCINE). L. A. SILBER and E. I. WOSTRUCHOWA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:239, 1931.

The previous communication dealt with the use of glycerin as an antidenaturing medium for the vaccine. Due to its locally irritating qualities, it was successfully replaced by saccharose and table sugar. (Fructose proved unsatisfactory.) The thermal death points of *Bacterium paratyphosum* B, *Bacterium abortum equi* and of other bacteria suspended in the sugars mentioned were raised. Mice immunized with vaccines of *Bact. paratyphosum* B and *Bact. abortum equi*, prepared with the sugars, showed a lower mortality and greater resistance to subsequent injections with the live organisms than when vaccines prepared with physiologic solution of sodium chloride were used. About 500 persons immunized with *Bacterium typhosum* vaccine, prepared as described, showed less local and general reactions and as good an antibody response as an equal number treated with a regular formaldehydized vaccine.

I. DAVIDSOHN.

ATTEMPTS TO IMMUNIZE RABBITS AGAINST TUBERCULOSIS. K. W. JOETTEN and W. PFANNENSTIEL, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:250, 1931.

Rabbits were treated intravenously with small, increasing doses of salt suspension of dried tubercle bacilli of human type, having a low virulence for rabbits and not infectious for guinea-pigs. A considerable number of them showed an increased resistance to tubercle bacilli and a few were fully protected. Immunization with B C G was not more efficient than with human strains, but when administered to rabbits with old chronic lesions B C G seemed to be beneficial. In none of the experiments did B C G show any advantages as compared with the human strains. The Friedmann tubercle bacilli seemed to behave in a manner similar to B C G, but were somewhat less effective. Inhalation of human tubercle bacilli of a low virulence for rabbits produced a gradually increasing resistance against virulent bacilli. After about three hundred days the effect of the immunization began to abate.

I. DAVIDSOHN.

THE SEPARATION OF HEMOLYTIC ANTIBODIES FIXED BY KAOLIN AND THEIR AMBOCEPTOR FUNCTION. CASPAR TROPP, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:289, 1931.

The hemolytic amboceptor removed from diluted antisheep hemolysin by kaolin could be separated from the latter with hundredth-normal sodium hydroxide. The amount recovered was directly proportionate to the original concentration of the amboceptor. Room and incubating temperature were more favorable than icebox temperature, but only if short periods (five minutes) were used. Treatment with physiologic solution of sodium chloride did not remove the hemolytic antibody from the kaolin even at 45 C. Kaolin loaded with amboceptor exerts hemolytic action after the addition of complement. The hemolytic tendency of the kaolin proper is eliminated by the added guinea-pig serum, and its anticomplementary effect is met by increasing the dose of the complement.

I. DAVIDSOHN.

COMPLEMENT-FIXATION EXPERIMENTS WITH ALCOHOLIC CANCER EXTRACTS AND WITH OTHER SYNTHETIC ANTIGENS. M. EISLER and J. JACOBSON, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:301, 1931.

Cholesterolized alcoholic extracts of cancerous tissues gave positive complement fixations with 50 per cent of serums from cancerous patients and with 35 per cent of various other noncancerous conditions. A pure cholesterol antigen, containing from 3.5 to 7 times as much of that substance as was present in the former, gave 55 per cent fixation in cancerous and 33 per cent in noncancerous conditions. There was no regularity in the behavior of the serums with both antigens. Serums from syphilitic and pregnant noncancerous patients reacted frequently with cancer extract but rarely with pure cholesterol. Extracts of normal organs did not react. Cholesterol and mixtures of palmitic and oleic acids and cholesterol were antigenic. Physicochemical reactions between the serum globulin and the antigen, but not specific cancer antibodies, are probably responsible for the phenomenon of the complement-fixation.

I. DAVIDSOHN.

TWO KINDS OF PRECIPITINS IN DIPHTHERIA IMMUNE SERUM. E. HOEN and L. TSCHERTKOW, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:325, 1931.

The presence of two separate precipitins (the antibacterial and the antitoxic) is confirmed with the ring precipitation method of the authors. The bacterial precipitins were of a considerably lower titer and independent of the antitoxic qualities, which were parallel to the titer of the antitoxic precipitins. The two could be separated from each other by absorption, without influencing the titer of the antitoxin. Mixing of unheated immune serum with another heated for fifteen

minutes at 65 C. removes its precipitating ability, as was shown by Schmidt and Scholtz, but with the author's method the antitoxic precipitins could be demonstrated.

I. DAVIDSOHN.

BACTERIOLOGIC INVESTIGATIONS OF A STRAIN OF TUBERCLE BACILLI ISOLATED FROM THE LUNGS AND LYMPH NODES OF A CHILD VACCINATED WITH BCG. A. ARIZTIA, J. ORELLANA and SÓTERO DEL RIO, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:370, 1931.

A male infant was given three doses of BCG by mouth on the sixth, eighth and tenth day after birth. The child died at the end of the second year, having suffered during life from congenital syphilis, bronchopneumonia and varicella. Post-mortem examination revealed tuberculous lesions of the lungs and mediastinal lymph nodes. The source of the tuberculous infection was traced with great probability to the BCG. Guinea-pigs into which suspensions of the pulmonary and lymphatic tissues were injected developed typical tuberculous lesions, from which tubercle bacilli virulent for rabbits but not for cattle were isolated. They were neither of the human nor of the bovine type, but behaved exactly like the BCG strain of increased virulence isolated by Hormaeche.

I. DAVIDSOHN.

FURTHER OBSERVATIONS ON THE RELATION BETWEEN THE WASSERMANN REACTION AND THE BLOOD GROUPS. ANNA SCHAPIRO, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:381, 1931.

The previously reported observation (*Ztschr. f. Immunitätsforsch. u. exper. Therap.* **64**:1, 1929) that in patients of group O  $\alpha\beta$  the positive Wassermann reaction turns negative sooner than in the other groups is explained by the fact that the titer of the natural antishcep hemolysin is higher, and that of the complement lower, in that group than in the others. Following a thorough absorption of the natural hemolysin, the negative reaction in many of these serums became positive.

I. DAVIDSOHN.

## Tumors

TUMOR IMMUNITY. THOMAS LUMSDEN, *Am. J. Cancer* **15**:563, 1931.

The mechanism of resistance to the implantation of malignant neoplasms has been investigated by numerous workers with contradictory results. Lumsden states that he "began the study of tumor immunity with a mind untrammelled by too deep a knowledge of the literature . . . and taking nothing for granted has investigated independently a large number of crucial points." As a result of his multiple experiments he reached the following conclusions: Antibodies lethal to cancer cells, but harmless to normal tissue cells, can be produced. When an implanted tumor already established in the body is gradually destroyed by the injection of antiserum or of formaldehyde into it, active immunity against the tumor is induced by a mechanism that may be called autovaccination.

Lumsden finally stated that the most hopeful means of attaining a "cure" of cancer appears to be (1) to inject antiserum into the primary cancer (or the arteries supplying it), localizing it there by constriction or by epinephrine; (2) thereafter, if necessary, to complete by operation or by irradiation the local destruction of the tumor.

B. M. FRIED.

PRIMARY LIPOSARCOMA OF BONE. F. W. STEWART, *Am. J. Path.* **7**:87, 1931.

In reporting these three cases of tumor of bone as cases of liposarcoma, I am fully cognizant of the fact that I am in doubtful territory. In no case has it been possible to trace the actual origin of the tumor to fat cells, although in one instance

the inflammatory fatty changes in the marrow apart from the tumor were suggestive. The conclusions drawn rest entirely on circumstantial data: the resemblance of the tumor cells to fat cells, the presence of large fat droplets in one case, the general resemblance to fat cells in another, although no specific fat stains are available, and the xanthomatous droplets in the second case; the peculiar clinical course in all three instances, namely, a suggestion, at least, of a multiplicity of bone lesions, which recalls the behavior of certain of the liposarcomas that I have observed in the soft parts; the lack of evidence of a primary epithelial origin, and an appearance inconsistent with primary tumors of bone of known types. That the disease is more or less of a clinical entity may be surmised from the fact that all three of the tumors either were multiple in bone or else showed a pronounced tendency to metastasize to other bones, and from the fact that the two treated by irradiation both proved to be radiosensitive. The bony tumors regressed markedly in the one case, and the pulmonary metastases were long held in check in the other. This radiosensitivity is inconsistent with either a primary tumor of bone of an osteogenic variety which even remotely approaches these liposarcomas in structure, or a metastatic adenocarcinoma, but it is not inconsistent with certain liposarcomas that I have observed in the soft tissues. It is of further interest from the clinical standpoint that whereas in osteogenic sarcoma cranial metastases are rather uncommon, in two of the three tumors that I have regarded as liposarcoma cranial involvement occurred.

AUTHOR'S SUMMARY.

CONGENITAL RHABDOMYOMA OF THE HEART. L. FARBER, *Am. J. Path.* 7:105, 1931.

Only two instances of this rare tumor have been reported previously in American literature. In Farber's case, which occurred in a girl, aged 6 months, there was also cerebral tuberous sclerosis. The entire literature on congenital rhabdomyoma is reviewed and discussed.

PRIMARY NEOPLASM OF HEART VALVE. C. F. BRANCH, *Am. J. Path.* 7:157, 1931.

A papillary branching fibroma of the tricuspid valve is described. The tumor, like the valve, contained no blood vessels. Every papillary stalk of the tumor was composed of a single or a compound core of dense collagen fibrils surrounded by loose connective tissue, outside of which was a layer of homogeneous material covered by endothelium. The tumor seemed to show a gradual growth and transformation of fibroblasts, starting in the homogeneous layer and ending in dense fibrous tissue. A similar homogeneous layer occurred beneath the endothelium lining the valve.

AUTHOR'S SUMMARY.

CHONDROSARCOMA WITH INTRAVASCULAR GROWTH AND TUMOR EMBOLI TO LUNGS. S. WARREN, *Am. J. Path.* 7:161, 1931.

A case of chondrosarcoma with extensive intravascular growth and tumor emboli in the pulmonary circulation is described.

AUTHOR'S SUMMARY.

INTESTINAL ADENOMA IN SWINE. H. E. BIESTER and L. H. SCHWARTE, *Am. J. Path.* 7:175, 1931.

The characteristic proliferations of bile ducts in coccidiosis of the liver in rabbits, the adenomatous formations in the equine stomach due to nematodes, the adenomas in bilharziasis and the characteristic columnar cell-lined adenomatous proliferations described in connection with infestations of the lungs by worms and pneumonia of sheep all suggest a relationship between intestinal adenomatous proliferations and destructive processes followed by epithelial regeneration, as against their interpretation as independent tumors arising from isolated or misplaced embryonic rudiments.

FROM AUTHORS' SUMMARY.

QUADRUPLE INOCULATIONS OF ADENOCARCINOMA. J. J. BITTNER, *J. Cancer Research* **14**:466, 1930.

Transplants of an adenocarcinoma were inoculated at four sites simultaneously in each of 462 mice. The results supported the genetic theory of transplantation. The organism responds alike to multiple transplants from the same tumor tissue. The physiologic characteristics of both the host and the tumor tissue are controlled by intrinsic genetic factors.

B. M. FRIED.

THE IODINE CONTENT OF THE BLOOD OF PATIENTS SUFFERING FROM CANCER. FRANK S. FOWWEATHER, *Brit. J. Exper. Path.* **11**:400, 1930.

There is no uniform reduction of the iodine of the blood in cases of cancer. Of twenty-four cases examined, only eleven showed values for the iodine of the blood below what may be considered as normal. This failure to find a constant association between a low iodine content of the blood and cancer is definite evidence against the view that a deficiency of iodine is a cause of cancer. While in cancer there may be a tendency toward a lowered iodine content of the blood, there is at least as much justification for assuming that the reduction is the result of the cancerous condition as there is for assuming that it is the cause of this condition. Further, since a reduction of the iodine of the blood was indicated in rather less than half of the cases examined, the determination of the iodine content of the blood as a diagnostic test for the presence of a malignant disease can have no value.

AUTHOR'S SUMMARY.

THE INFLUENCE OF VITAL STAINING ON INDUCED RESISTANCE TO THE GROWTH OF TRANSPLANTABLE TUMOURS. R. J. LUDFORD, *Brit. J. Exper. Path.* **12**:45, 1931.

By vital staining with trypan blue it is possible to break down the immunity to tumor transplantation induced by the inoculation of an emulsion of embryonic skin.

AUTHOR'S SUMMARY.

A CASE OF PRIMARY ADAMANTINOMA OF THE TIBIA. A. H. BAKER and L. M. HAWKSLEY, *Brit. J. Surg.* **18**:415, 1931.

A tumor occurred in the lower part of the left leg following an injury in a patient, 46 years of age. A portion of the tibia was removed and showed an infiltrating epithelial neoplasm in a fibrous tissue stroma containing giant cells. There were occasional whorls of stellate cells with an outer layer of columnar cells. The growth had the histologic character of adamantinoma, the stellate cells representing the enamel pulp and the columnar cells the ameloblasts of the embryonic enamel organ. There was no evidence of a formation of enamel. The epithelial tissue was accounted for on an embryonic basis. The authors have found only one similar case, that of Fischer (*Frankfurt. Ztschr. f. Path.*, 1913).

M. C. PORTERFIELD.

HEMENDOTHELIOMA OF SPLEEN AND BONE MARROW. C. G. PAINE, *J. Path. & Bact.* **34**:139, 1931.

A malignant tumor of the spleen and bone marrow with a metastasis to the liver is described. Histologically it appeared to be a hemendothelioma.

AUTHOR'S SUMMARY.

ASTROCYTOMA OF THE CEREBRUM SHOWING EXTENSIVE INVOLVEMENT OF THE OPPOSITE CEREBRAL HEMISPHERE. E. A. LINELL and K. G. MCKENZIE, *J. Path. & Bact.* **34**:195, 1931.

An astrocytoma that had shown clinical evidence of its presence for over nine years before the death of the patient was submitted to detailed histologic examina-

tion. This revealed widespread infiltration by tumor tissue far beyond the macroscopic limits of the growth. The greater part of the tumor as delimitable by the naked eye was seen histologically to correspond to its degenerating portion. Although the growth had spread from the left into the right cerebral hemisphere, little clinical evidence of its presence in the latter had been shown, even though pathologic changes were noted in nerve fibers entering this part of the tumor. This case raises the question of the value of deep roentgen therapy for this type of tumor. Bailey, Sosman and van Dessel (1928), in an analysis of a large number of cases of glioma that were submitted to roentgen therapy, concluded that the astrocytomas were among those groups least favorably influenced by this form of treatment. Recent work on the effects of radium on normal tissue of the brain by Carmichael and Ross (1930) has shown that this agent can produce well marked astrocytic proliferation not only around the area destroyed by the radium, but also within the area of the lesion. There seems to be a possibility, therefore, that the rate of growth of an astrocytoma may be stimulated by this form of therapy. In this connection it is perhaps worthy of note that treatment by x-rays produced no definitely beneficial effect in this case.

AUTHORS' SUMMARY.

METASTASIS OF THE "BENIGN" GIANT-CELL TUMOR OF BONE. S. C. DYKE, *J. Path. & Bact.* **34**:259, 1931.

A case of "benign" giant cell tumor of bone is recorded, in which metastases of the same structure as the primary tumor were demonstrated in the scalp, lungs, kidneys and mediastinal and peritoneal lymph nodes; there was evidence of metastases in the ribs and the spine, but this was not proved histologically. The history of the case extended over five years. It is concluded that this tumor, unless completely eradicated, is liable in the course of time to assume malignancy of a high order.

AUTHOR'S SUMMARY.

TUMOR IMMUNITY: THE EFFECTS OF THE EU- AND PSEUDO-GLOBULIN FRACTIONS OF ANTI-CANCER SERA ON TISSUE CULTURES. THOMAS LUMSDEN, *J. Path. & Bact.* **34**:349, 1931.

When the euglobulin and the pseudoglobulin fractions of an anticancer serum are isolated, the euglobulin fraction contains all the antibodies that are specifically toxic to cancer cells; it also contains any heterotoxins that have escaped destruction during the process of fractioning. The pseudoglobulin fraction contains the antispecific bodies. By fractioning anticancer serum as described, it can in effect be concentrated tenfold, since the euglobulin fraction is ten times less toxic to mice than the equivalent quantity of anticancer serum from which it was made, although it has lost none of the original toxicity to the cancer cell. It is considered that the experiments described demonstrate beyond reasonable doubt the existence of antibodies having a specific affinity for cancer cells.

AUTHOR'S SUMMARY.

THE CITELLI-PIAZZA REACTION IN MALIGNANT TUMORS. P. SARCO, *Arch. di pat. e clin. med.* **10**:98, 1930.

All except three of eighteen cases of cancer showed a distinct hemoclastic crisis after the injection of fresh tumor extract. The author recommends this reaction for further study on account of its high positive percentage.

EMMERICH HAAM.

THE STIMULATING EFFECT OF ARSENIC ON CHICKEN SARCOMA. L. CALIFANO, *Riv. di path. sper.* **6**:113, 1930.

Arsenic acid stimulated the growth of a previously slowly growing Rous sarcoma. It also changed the physical nature of the tumor, making it softer and

more vascular, with an inclination to liquefaction in the center. The mode of the action of the arsenic is not clear.

EMMERICH HAAM.

THE GROWTH OF TUMORS IN TISSUE WITHOUT NERVES. E. BRESCIANO and E. CANCELLARA, *Riv. di path. sper.* **6**:119, 1930.

To test the influence of nerves on the development of tumors a measured quantity of an emulsion of a tumor was injected into the muscles of a chicken's thigh from which the sciatic nerve had been removed. At the same time the other thigh with intact sciatic nerve received an injection of an equal quantity of the emulsion of tumor. No difference in the growth of the implanted tumor tissue was observed.

EMMERICH HAAM.

THE EFFECTS OF DYES ON TAR CANCER IN MICE. J. FORSMAN, *Acta path. et microbiol. Scandinav.* **8**:16, 1931.

The injection of solutions of a large number of dyes had no effect either favorable or unfavorable on tar cancer in mice.

### Medicolegal Pathology

CRIMINAL ABORTION. P. FRAENCKEL, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:405, 1931.

In criminal abortion, the main fact to be proved is whether there is a pregnancy. Therefore, a microscopic examination of the uterus should never be omitted. Gravidity changes of the uterine muscles and blood vessels, as well as the invasion of the myometrium by chorion, can be found in spite of a complete removal of the mucous surface. There are two main types of general infection following criminal abortion: (1) septicemia without any thrombotic or suppurative processes, with septic swelling of the spleen, cloudy appearance of parenchymatous organs, septic icterus with hemolytic processes, etc.; (2) the thrombophlebitic (pyemic) type, with metastases, peritonitis, etc. In the first group, the anatomic interpretation as to the portal of entry, when there are no gross or microscopic lesions of the genital organs, may be extremely difficult. Presence of bronchopneumonia may complicate the situation, since it might be diagnosed as the primary cause of the septic abortion. In instances of infection of the embryonic sac, one may find only a septic metritis, without evidence of the septic endometritis of induced abortion. Important are the concomitant changes of the ovaries, which may contain confluent abscesses due to suppuration in follicular cysts; often a rapid softening and complete destruction of the ovary may follow. One may be deceived and assume that these changes are primary or contributory causes of the abortion. The progress and the route of the infection can be more clearly followed in the thrombophlebitic form, as the thrombotic process is a reliable guide; it leads one from the surface of the uterine cavity, through the uterine wall and its venous plexus, into the parametrial plexus, to the hypogastric vein. Thrombophlebitic involvement of the spermatic (ovarian) vein, often unilateral, is common and may reach the renal vein and extend even into the inferior cava. If the myometrial thrombotic process suppurates, one finds minute intramural abscesses, well discernible on the cut surface. In the central nervous system, there may be subpial miliary abscesses, and the brain tissue may contain circumscribed necrosis, due to septic emboli. Peritonitis following abortion occurs also, on account of migration of micro-organisms through the uterine wall without primary involvement of the genital organs. In instances of purulent accumulation in Douglas' pouch, the external layers of the uterus may show deep septic softening, which should not be misinterpreted as signs of injury. If intra-uterine injections were used, the ovaries often contain abscesses, and there may be pus in the distal part of the tubes; the entire tube, however, is rarely involved by a uniformly developed sup-



purative process. Infections with the gas bacillus are not uncommon and are observed usually in connection with intra-uterine injuries. The uterus appears greatly distended (physometra), and tears occur, which one may erroneously believe to have been produced mechanically. Under normal conditions, this micro-organism is found in the genital canal of about from 30 to 40 per cent of adult women. One may occasionally hesitate to diagnose a case of gas bacillus infection, believing that one is dealing with a cadaveric emphysema, but the presence of icterus, of pigment deposits due to hemolysis, of methemoglobin casts in the kidneys and of necrotic areas at the site of the placenta will safeguard one against a wrong conclusion. A complete skeletization of a 6 months old fetus occurred, in a case of gas infection, within twenty-two hours, and in another case within from twenty-seven to thirty-six hours. Acute verrucous endocarditis may be seen at the end of the first week, in the form of delicate gray-red thrombotic efflorescences, and if present with the genital infection, is undoubtedly secondary. Otherwise, endocarditis leads rarely, if ever, to suppuration of the uterus. In 17 per cent of 209 abortions, injuries to the uterus were found. Perforations in the cornua of the uterus are usually produced by the curet in the hands of a physician. Injuries of the cervical canal are rather common; they may heal completely before death or may show, on microscopic examination, inflammatory (purulent) changes. In instances of sudden death following abortion, one should be on the alert for air embolism and thoughtfully employ the required autopsy technic. In decaying bodies, a positive diagnosis of air embolism is impossible.

E. L. MILOSLAVICH.

ACUTE YELLOW ATROPHY OF THE LIVER FROM POISONING BY MOREL (HELVELLA ESCULENTA). UMBER, *Med. klin.* 1:947, 1930.

A woman, aged 40, became ill after eating morel. Icterus developed in severe form, and death occurred in coma on the ninth day. The autopsy revealed acute yellow atrophy of the liver in extreme degree. In light cases of poisoning by the morel mushroom there are symptoms and signs of injury to the hepatic parenchyma (K. Landé: *München. med Wchnschr.* 2:1615, 1930).

POISONING FROM FERROSILICON. A. JENELL and M. HAALAND, *Med. rev., Bergen* 47:145, 1930.

The article describes the poisoning, presumably by hydrogen phosphide, of seven passengers, three of whom died, on board a ship loaded with 75 per cent ferrosilicon. The symptoms were in the main dizziness, headache, vomiting, weakness and unconsciousness. Warm-blooded animals are killed in a short time by 0.2 per cent hydrogen phosphide, which may be given off by ferrosilicon. Both in poisoned animals and poisoned human beings the main changes are in the lungs: congestion, edema and ecchymosis. Hydrogen phosphide is decomposed as it reaches the lungs by inhalation; consequently the chemical demonstration of the poison is practically impossible. Ten other instances of similar poisoning on board ship are mentioned; in one instance fifty persons were poisoned, with eleven deaths.

PECULIAR CADAVERIC CHANGES OF BRAIN. W. GERLACH, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 16:431, 1931.

Microscopic examination of the brain removed from a person who drowned and whose body was floating in water for four weeks disclosed accumulations of cellular elements in the leptomeninges, and a chronic meningitis was assumed. Gerlach reexamined the tissues and showed that the round cells did not surround the blood vessels entirely, being present only on their upper or free side, but never toward the surface of the brain. No cellular elements were evident in the depths of the gyri. Since the cerebellum and peduncles were decayed, he assumes that these accumulations were cadaveric artefacts due to the transportation of decayed masses as a result of continuous moving and shaking of the body in the water.

E. L. MILOSLAVICH.

A SIMPLE METHOD OF TYPING DRIED BLOOD. FRANZ JOSEF HOLZER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:445, 1931.

The description of the technical procedure should be read in the original. The results obtained show that receptors A and B are exceedingly stable. Their resistance is much higher than that of the agglutinins and permits group determinations not only on minute traces of blood, but also on old and dried spots of blood. Errors may occur on account of an unspecific union or owing to nonunion. The detection of blood groups by this method gives correct results in 90 per cent of the cases. Some faulty reactions may occur, however, in this as in any biological test.

E. L. MILOSLAVICH.

GAS ANALYSIS OF PUTREFYING LUNGS. B. MUELLER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **16**:459, 1931.

Analysis of the oxygen content of the lungs was recently recommended by Dyrenfurth as a new method to establish whether a new-born infant, the body of which has putrefied, was born alive or did not breathe. The author found that the oxygen content of the alveolar air in decayed lungs that have breathed, regardless of the temperature in which they are kept, drops relatively very rapidly, and after from four to six days amounts to only from 2 to 2.5 per cent. In instances of advanced putrefaction, in which the lung tissue becomes partly disintegrated and porous, the atmospheric oxygen may penetrate these areas. Analysis of lungs that did not breathe disclosed approximately the same amount of oxygen as those that had breathed. The gas analysis consequently is not reliable in the case of decayed lungs.

E. L. MILOSLAVICH.

INJURIES OF THE HEAD CAUSED BY BLUNT INSTRUMENTS. WALCHER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17**:22, 1931.

In certain cases of injury to the head the question may arise whether the injury is the effect of a blow or of a fall. In examining it, occasionally one may clearly see the imprint of a blunt instrument or recognize the contact surface of the impact object. If the injury is found on the lateral aspect of the head, one should not omit to examine that side of the body, particularly its prominent areas, such as the shoulder, elbow, trochanter region, ankle, etc. Deep incisions are necessary, since bruises of the deeper lying structures, such as hemorrhages within the muscles, traumatic pocket formations within the soft tissues, etc., may be present without any evidence of injury to the skin. In instances of a fall on the back of the head, hemorrhages may be found in both iliopsoas muscles, apparently due to abrupt, intense contractions of these muscles during the balancing of the body. A fall on smooth and even ground will, as a rule, produce bruises at the hat-brim level; all injuries above this line are to be suspected of having been inflicted by an assailant. Linear fractures, and also comminuted fractures, may develop in a simple fall on a paved road, for instance in intoxication, particularly in older people. A depression of the fragments may occur if a projecting area or object, such as a small stone, was present at the site of the impact. A vertical linear fissure through the occipital bone indicates a fall on the back of the head. Terrace-like, sloping fractures are observed not only in instances of a blow with a blunt instrument, but also in a fall on an edged surface. Pinching of hair between the fractured bone fragments is best seen on macerated bone and occurs most often in cases of blunt injuries. Large pocket formations indicate action of a moving force, for instance, an automobile, and are never met with in a simple fall. Microscopic examination of abraded or excoriated surfaces or of the edges of the wound may reveal the presence of foreign bodies and be of particular significance. Contrecoup-injuries of the brain may also develop in instances of fall and were found thirty-six times in eighty-four cases.

E. L. MILOSLAVICH.

DETECTION OF CARBON MONOXIDE IN EXHUMED BODIES. WALDEMAR WEIMANN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17:48**, 1931.

Presence of carbon monoxide can be proved even in decayed bodies, as, for instance, in cadaveric transudations in the pleural cavity or in mushy parts of the brain. In two cases, forty-five and fifty days, respectively, after death, chemical and spectroscopic examinations of the pulp of the spleen and of the lungs were successful.

E. L. MILOSLAVICH.

POTASSIUM CHLORATE POISONING. Z. MORGENSTERN and M. AWDEJEV, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17:51**, 1931.

Two cases are described, in which intravenous injections of Ringer-Locke solution containing minute amounts of potassium chlorate were administered. Typical clinical symptoms of potassium chlorate poisoning appeared, and death followed in a few days from anuria and methemoglobinemia. The cadaveric hypostasis was of a purple-blue color.

E. L. MILOSLAVICH.

### Technical

SUPRAVITAL STAINING WITH SILVER AMMONIUM CARBONATE. F. A. MCJUNKIN, *Am. J. Path.* **7:131**, 1931.

Silver ammonium carbonate may be used to mark living cells supravitaly. By supravital staining with silver ammonium carbonate the origin of the monocytes is seen to be from the silver-marked portion of lymphoid tissue. The histiocytic tissue of the sinusoidal organs also reacts to supravital silver, but the response is unlike that of the monocytes. The two component parts of the so-called reticulo-endothelial system are so unlike in function and structure that they should not be grouped together. The histiocytic tissue of the sinusoidal organs, consisting mostly of anchored cells, functions chiefly as a fixed tissue. On the other hand, the monocytes, originating in the lymphoid tissue, are a normal element of the circulating blood and may be concentrated quickly in any tissue or cavity.

AUTHOR'S SUMMARY.

GALACTOSE IN TESTS OF THE FUNCTION OF THE LIVER. HARRY SHAY, EUGENE M. SCHLOSS and MILTON A. BELL, *Arch. Int. Med.* **47:391**, 1931.

Galactose seems to be suitable for the testing of the function of the liver because it is obtainable in pure form, is readily absorbed from the digestive tract, is converted into glycogen by the liver with some difficulty as compared with other sugars (dextrose and fructose), is practically not utilizable by any tissues other than the liver, and because, after it reaches the general circulation, it is excreted in the urine regardless of either the state of the renal excretory mechanism or the activity of the endocrine glands.

FROM AUTHORS' SUMMARY.

THE SEROLOGY OF SYPHILIS. H. EAGLE, *J. Exper. Med.* **53:605 and 615**, 1931.

The discovery that there are many substances with the sensitizing properties hitherto believed peculiar to cholesterol and its derivatives, and that sensitizer added to antigen in quantities many times those currently used continues to increase the complement-fixing efficiency of the antigen without giving falsely positive reactions, has made possible the preparation of an antigen much more sensitive

than any hitherto available for use in the Wassermann reaction. Dry powdered beef heart muscle, 100 Gm., is extracted with ether, 500 cc., for fifteen minutes at 37 C., with shaking. After filtration with suction, the ether filtrate is discarded. The powder is then dried and extracted for from three to five days with 500 cc. of 95 per cent alcohol, with intermittent shaking. The mixture is filtered, and the moist powder washed on the filter paper with two portions of alcohol, each 100 cc. The alcoholic filtrate and the washings are combined and evaporated on the steam bath to from 250 to 300 cc. Cholesterol (0.8 per cent) and sitosterol (0.6 per cent) are then added, and dissolved at from 65 to 75 C. The excess sensitizer that crystallizes out on cooling is dissolved just before use by immersing the antigen in a bath at 56 C. for a few minutes. The antigen is diluted by pouring saline solution into it rapidly. A dilution of 1:40 is recommended for use in water bath fixation (one-half hour at 37 C.), as well as for the short icebox fixation (four hours at 8 C.), and a dilution of 1:120 for use in the overnight icebox fixation (from sixteen to twenty-four hours at 8 C.), as being well beyond the anticomplementary range, the anticomplementary dilutions being 1:5 and 1:25, respectively. There is reason to believe that this antigen possesses almost the maximum sensitivity obtainable. The method of preparation insures that the antigen is almost saturated with antigenic lipoids, and more sensitizer could not be added without increasing the turbidity of the dilution in saline solution to a point where it would interfere with the reading of hemolysis. Any further improvement must await the discovery of better sensitizers. Preliminary experiments indicate that this new sensitizer, sitosterol, will find an immediate application, not only in the Wassermann reaction, but also in a more sensitive flocculation test to be described.

Serum, in concentrations greater than 1:25, causes a marked inhibition of complement-fixation in general and of the Wassermann reaction in particular. The serum protein is probably adsorbed by the colloiddally dispersed lipid-reagin complexes, forming a protective film that prevents the fixation (adsorption) of complement. This inhibition explains the zone phenomenon in complement-fixation: a weakly positive serum may give a completely positive reaction in, e. g., a dilution of 1:5, and yet appear completely negative when tested as whole serum. The greater sensitivity of the icebox test is due to the less marked inhibition by serum at lower temperatures, to the prolonged period of incubation, making for greater specific fixation, to a more marked nonspecific destruction of complement by antigen and to a spontaneous deterioration in the longer icebox test. Because of the inhibition by serum in high concentration, a quantitative Wassermann technic involving the use of graded quantities of serum is worthless when carried out at 37 C. Even the icebox test, which is less susceptible to this inhibiting effect, yields a positive reaction with whole serum only when the circulating reagin exceeds a surprisingly high threshold (six to ten times the quantity that could be detected in dilute serum). It is well known that a negative Wassermann reaction, even by a very sensitive test, does not exclude syphilis; it now appears that a negative Wassermann reaction does not exclude circulating reagin.

#### AUTHOR'S SUMMARIES.

COLON-AEROGENES ORGANISMS. F. O. TONNEY and R. E. NOBLE, *J. Infect. Dis.* 48:413, 1931.

The practical advantages of direct plating in cyanide-citrate agar are: The method is simple and yields results in forty-eight hours. *Bacillus coli* and *B. aerogenes* may be separated and their numerical relationship established. A more accurate index is obtained than in liquid mediums. The direct count is not obscured by overgrowths and not interfered with by other lactose-fermenting organisms, such as *Clostridium welchii* or *B. aerosporus*. The "pour plate" method with deep colonies insures the isolation of pure cultures more easily than the surface streak method.

FROM AUTHORS' SUMMARY.

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, Feb. 26, 1931*

LEILA CHARLTON KNOX, *President, in the Chair*

### CONGENITAL TRACHEO-ESOPHAGEAL FISTULA CAUSED BY AN ABERRANT DUCTUS ARTERIOSUS. HENRY RASCOFF and MENDEL JACOBI.

On the day of its birth a full term infant with no external developmental defects showed suffocative symptoms with each feeding and returned all food through its nose. Esophagoscopy and roentgenologic examination confirmed the clinical diagnosis of esophageal stenosis at the sternoclavicular junction and tracheo-esophageal fistula. The child died of diffuse bronchopneumonia within two weeks after birth.

Autopsy revealed a large blood vessel of the same caliber as the aorta arising from the descending aorta and running upward and medially to the right, then forward and downward to enter the posterior surface of the left pulmonary artery near its origin. In its course the aberrant vessel lay dorsal to the tracheo-esophageal fistulous tract and just below and slightly ventral to the lower end of the stenosed upper esophageal pouch. Behind the vessel lay a fibrous cord connecting the two portions of the esophagus; no epithelial, muscular or cartilaginous elements were present in this cord.

The anomalous tracheo-esophageal formation was interpreted as having been caused by the aberrantly situated and unusually large-calibered ductus arteriosus. This is in accord with the view of Keith that external pressure factors (chiefly of vascular origin) are responsible for the anomaly in question. The supporting cases of Harris and of Shellshear and Anderson were reviewed. The opposing view of Lewis was discussed.

#### DISCUSSION

ALEXANDER H. ROSENTHAL (by invitation): While working as an assistant in the department of pathology at Mount Sinai Hospital several months ago, I had the privilege of helping at postmortem examinations in two cases of congenital esophageal atresia. These aroused my interest in the subject, and I went down to Baltimore to see if a study of the normal development of the trachea and esophagus at the time the separation takes place would not throw some light on the formation of this anomaly. As Dr. Rascoff and Dr. Jacobi stated, the article by Plass was the most complete up to 1919. He reviewed 146 cases. I have reviewed 80 cases of tracheo-esophageal fistula with congenital atresia of the esophagus since his review. In the 6 or 7 cases mentioned by Dr. Rascoff, and in the 8 cases that are available to me, 5 from Mount Sinai Hospital and 3 from the Brooklyn Jewish Hospital, there has been no record of an arterial anomaly concomitant with this congenital anomaly.

In order to understand the development of this anomaly, I believe one must fix his attention on the stage in which the trachea separates normally from the esophagus. I am showing a photograph of an actual reconstruction of the entoderm and the arterial tree at this stage in a 4 mm. human embryo. One will notice ventrally a pulmonary anlage separating from a primitive esophagus in the manner outlined by Drs. Rascoff and Jacobi. If one examines this diagram (and I have seen the actual cross-sections of this embryo while working with Dr. Streeter), one sees how far the arterial arches, including the last, are from the primitive entoderm, which ultimately gives rise to the trachea and the esophagus. A striking fact is the constant morphology of this anomaly. There is always an upper

atretic sac and practically always a lower portion entering the trachea at or very near its bifurcation into the bronchi. It means then that this posterior portion of entoderm must be the site of the lesion, whatever that lesion may be. It seems a little far fetched, therefore, to assume that distant pulmonary vessels which ultimately may give rise to an aberrant ductus arteriosus should affect this separation at the time when it occurs. One gains the impression in reading Congdon's article from which this photograph was taken, and another written in 1926, that an anomaly in the formation of blood vessels is not the cause of anomalies of any system, including this one, but is rather in turn the result.

Ysander reported an interesting embryo about 8 mm. long in which this anomaly was present. This is the earliest embryo showing this type of anomaly, the only other one measuring 18 mm. If it is true that aberrant blood vessels cause this anomaly, one would expect a description of such blood vessels in the very complete paper by Ysander, and he makes no mention of any such anomalous vessels, but ascribes the formation to pressure by means of a large cardiac anlage. Cross-sections of the embryo shown in this photograph and those of the same age and a little later, well within the period within which this separation takes place, did not demonstrate to me any vessels that might possibly influence the development of such an anomaly at this stage or at a slightly later stage at which it must occur.

Mackenzie reported a father all of whose children, from three wives, presented this anomaly. This marked constancy of the malformation, and also Ysander's interesting embryo, in which the malformation was discovered bilaterally at this very early stage, seem to suggest that its development is due to an early fundamental change in the entoderm rather than to primary vascular changes.

The most recent work on this subject has been done by the German investigators. Zausch, a pupil of Benecke, advanced Benecke's mechanical theory of the development of the malformation, and a little later work was done by Schmitz and also by Ysander. They all had essentially the same idea, namely, that the anomaly resulted from pressure of a large cardiac anlage on this primitive esophageal tube. As was stated by Dr. Rascoff, this tube consists dorsally only of a thin layer of cells and ventrally of three or four layers of cells. They conceived of a large cardiac anlage pressing on this tube and effecting the greatest tension dorsally. This causes either rupture or at least shifting of the cell mass at this site, resulting in atresia.

These theories, while suggestive, are not borne out by study of embryos actually in this stage of development. First, the anlage of the heart not only is normally large, but, even more than is demonstrated in this figure, it is a definite distance from this tube. Second, the curvature which they speak of and represent diagrammatically as being very extensive simply does not exist. This slide shows an actual construction of the entoderm, and you see that there is very little curvature here. Third, it is difficult to see why pressure ventrally in such a manner should cause a malformation of the esophagus and should leave the trachea, which is nearer to it, to develop apparently normally, as it does in every instance. Therefore, it appears that tension due to a large cardiac anlage is not the factor causing this anomaly. If one notices here, one sees that these arterial arches take a course ventral to the esophagus and meet the dorsal aortae laterally and only slightly dorsally. I bring this up to show that the position of these vessels, which are conceived to come down later, pursuing as they do a ventral course at the time the separation is taking place, makes it seem unlikely that they cause the malformation.

Apparently, then, the anomaly is the result of a very early change. In one of the embryos of Dr. Streeter's collection I followed the entoderm (which was later to give rise to the esophagus at the region of the separation of the trachea and which must be the center of the pathologic changes in these cases) back to the stage in which the neurenteric canal seemed to open into this region of the entoderm (demonstrating this diagram). Possibly this relationship might have something to do with this early change, but whether it does or not, the change is very probably an early one, possibly on a genetic basis, if Mackenzie's citation is true, and not on the basis of primary cardiovascular changes.

I believe that arterial anomalies in this condition are far more frequent than have been described. Probably they are not always observed. Such anomalies must represent vessels that have had an abnormal course laid down for them and are aberrant because of primary changes in this entodermal system; they are the result of this malformation rather than the cause of it.

MENDEL JACOBI: From the discussion I gather that the chief points of objection to the interpretation as given by Dr. Rascoff are, first, that the vessel in question has no means of getting to the dorsal aorta behind the esophagus, as we noted in our diagram, and second, that at the stage in which the tracheoesophageal separation takes place, the vessel is not in relation to this particular anlage. In answer to the first objection, our own specimen and those of Harris and Shellshear and Anderson are sufficient to prove that the vessel is there. The exact embryologic explanation I cannot give, and I cannot see that embryologists are at all certain of the exact embryogenesis of all the vessels. In fact, in descriptions of the development of the various vessels, one regularly comes across the statement that aberrant capillary loops are frequently present in the course of the vessels, and this in nearly every vessel from which the numerous adult vascular anomalies may arise. This point I shall touch on in somewhat greater detail later.

In connection with the second objection, that of the relation of the vessel to the anlage, I should like to call attention to the fact that these vessels do not start as definite vessels, but as a capillary plexus, from which the adult vessels apparently develop by marked enlargement of certain of these capillary vessels definitely, so that they become the future adult vessels, while the others of the original plexus atrophy. Such vessels have been described by Aeby, and after him, by Thoma, Ruge, Flint and Mall. They are not laid down haphazardly, but have a definite relation to the surrounding structures. Further, in the article from which Dr. Rosenthal shows his diagram, there is a statement by Congdon that in the interpretation of these vessels there is great difficulty in deciding at which stage it is to be considered a capillary. Discussing the sixth and disputed fifth aortic arches, the precursors of the future primitive pulmonary artery and ductus arteriosus, he notes that in these arches, of all the arches, aberrant island loops of capillaries are most frequent and most persistent. All the pulmonary arches, whether full formed or not, lie in deep grooves in the caudal pharyngeal complex (of Kingsbury); and at the stage of 35 somites, the fifth and sixth aortic arches have already formed. Congdon also described a downward extension of the pulmonic arches from the dorsal aorta, below this complex, while caudad from the aortic sac, he figured an elongated vessel extending backward beyond the level of the dorsal sprout to break up on the sides of the trachea. These vessels he described as well developed at the 5 mm. stage and as present before this, at a time when the lung bud anlage is still connected with the esophagus.

From the aortic sac plexus, a secondary plexus extends along the under surface and side of the common tracheoesophageal mass. These plexuses meet to form the pulmonic arches. Further, of all the arches, the pulmonic arches have most frequently and most constantly variable capillary island loops in their course, and these are most persistent. Also their relation to the fourth arch is variable. This may be far or near, or may even arise by a common stem, depending on the size of the postpharyngeal complex. Under these circumstances it seems not inconceivable that one of the aberrant capillary loops may enlarge sufficiently to cause pressure on the adjacent anlage and hence the anomaly in question.

This belief is given further support by the findings of Shaner. In 1921, he described in a turtle a sixth aortic arch lying craniolateral to the pharyngeal complex. From this vessel a spur ran caudomedially to the usual position of the human pulmonic arch. Such a vessel would well correspond with the one described by us.

ALFRED PLAUT: The speakers have gone so deep into details that probably no one who has not worked particularly in this line can add any, but I would like to make a general remark. It is astonishing how constant the picture of this malformation is. If an anomalous blood vessel causes the malformation, we should expect it to be always the same anomalous vessel and always in the same location and arrangement. Otherwise how shall we account for the constancy of the picture? Why do we never have separation of the esophagus and trachea in another way? Why do we always have this upper esophageal pouch and a connection of the lower part of the esophagus with the trachea? I saw one such malformation several years ago. I cannot claim that my examination was so careful as to exclude absolutely any anomaly of a blood vessel, but in view of the very early period in which the malformation must start, and in view of the one instance of familial occurrence, I would feel hesitant in ascribing this malformation to mechanical pressure by an anomalous vessel.

MENDEL JACOBI: I would like to bring up two other points. To ascribe so constant a malformation to a genetic basis on the ground of the one instance recorded by Mackenzie seems a bit precarious, because we have only the adult embryos; we have not the stillborn and the earlier embryos similar, perhaps, to the 18 mm. embryo described by Dr. Rosenthal, which may not have shown the anomaly found in the children mentioned by Mackenzie. The anomaly that seems to be an hereditary characteristic may have been an accident.

As far as the other point is concerned, I should like to point out that Congdon, in describing changes in vascular course and direction, not in this connection, but in connection with later embryos, makes the statement that these vessels may be very tortuous in the early embryonic stage, although in later adult form they are in a normal position and have left no evidence behind of their previous tortuosity. If one can carry the analogy a bit further, there may have been some such close vascular approximation, sufficient to cause cessation of the growth of the tracheo-esophageal anlage, the vessel later straightening as the body conformation assumed its mature form and leaving no trace of its previously tortuous course and proximity to the anlage. The tracheo-esophageal malformation has, however, already been formed.

#### SERUM AND PLASMA BILIRUBIN: A COMPARATIVE STUDY OF ONE HUNDRED CASES. HERMAN BOLKER and MENDEL JACOBI.

The study consisted of 100 consecutive and unselected simultaneous plasma and serum quantitative bilirubin determinations. The readings were subjected to statistical analysis. The results agreed exactly in over 70 per cent of the determinations. In the other 30 per cent, the average deviation of the differences in readings was of the same order as that of those obtained in quadruplicate determinations on the same specimens (five instances). These differences are within the range of experimental error and have no significance in clinical practice. It was shown that plasma and serum can be used interchangeably in such determinations. The time allowed for coupling to occur plays no part in the determinations. The results obtained corroborate experimentally the opinion of McNee and Keifer and refute the findings of Shay and Schloss. The results of the latter are critically reviewed.

#### THE OCCURRENCE OF MALIGNANT GROWTH IN PERSONS WITH RADIOACTIVE DEPOSITS. HARRISON S. MARTLAND.

For a number of years I have had the opportunity of following the clinical course of many of the original radium dial painters of the New Jersey plant in which occurred the occupational poisoning by radioactive substances in the watch dial industry first described by me and my associates in 1925.

These girls over long periods of time had accidentally ingested minute amounts of insoluble radium and mesothorium sulphates owing to their habit of both licking and pointing the brushes in their mouths in the process of painting watch dials.



It was possible for a dial painter to swallow from 7 to 150 micrograms of radioactive substance per week, most of which was quickly eliminated by way of the intestinal tract. Small amounts, however, reached the blood stream and were deposited as colloidal particles of radium and mesothorium sulphate in the organs of the reticulo-endothelial system, over 98 per cent being finally deposited in the bones. These skeletal deposits were similar in distribution to those of lead in industrial lead poisoning.

These deposits in the bones continually emitted, throughout the girls' lives, their characteristic rays, over 95 per cent of which were alpha. The small amount of beta and gamma rays was practically negligible. Thus, this was the first time that attention was called to the fact that human beings had been exposed to internal bombardment by alpha rays. The effects of internal alpha radiation are quite different and distinctly more irritative on the bones and blood-forming centers than those due to any form of external penetrative radiation.

The alpha particle—a double-charged helium nucleus expelled from the radium atom at a velocity approaching 80,000 miles per second—is one of the most potent and destructive agents known to science. Its disruptive effect is due to molecular collision and resulting ionization. This produces in the bone marrow an intense stimulative and compensatory hyperplasia, quite unlike, and more intense than that seen in any other disease. This I called the first stage of radiation osteitis. The marrow was dark red at autopsy and on histologic examination had the appearance of panmyelosis with very primitive stem cells. The ability to form cells of the granulocyte series had been lost, except for the production of enormous numbers of eosinophil myelocytes. The red cell production was greatly curtailed and was of the megaloblastic or embryonal type. Megakaryocytes were abundant.

This hyperplastic marrow was gradually replaced in various areas over the skeleton by very cellular fibroblastic tissue, which in many areas histologically mimicked sarcoma, there being abundant mitosis and hyperchromatism. This was designated as the second stage of radiation osteitis.

Finally, the marrow may become almost entirely replaced by old, acellular fibroblastic tissue with considerable decalcification of bone. This was interpreted as the final or healing stage of the radiation osteitis seen in these cases.

Many of the girls who showed the largest amounts of radioactivity, the radioactive substances being estimated at from 30 to 50 to 180 micrograms in their skeletons, died, usually from extensive necrosis of the jaws or from anemia or from a combination of both diseases, from one to five years after leaving their work as dial painters. The necrosis of the jaws was due to bacterial infection from the mouth superimposed on a preexisting radiation osteitis. The anemia was usually of the pseudo-aplastic type, the blood during life showing an anisocytosis with many macrocytes and a leukopenia with a tendency toward an agranulocytic blood picture. The hemorrhagic diathesis was not marked, as the blood platelets were usually not greatly diminished in number.

Following these early cases, no more girls died for some time. Occasionally, however, a former dial painter, who may have enjoyed several years of apparently good health, reported, presenting crippling and deforming lesions of the bones such as coxa vara, deformities of the spine, etc. Examination of these girls during life showed that their bodies contained from 10 to 20 micrograms of radioactive substances—chiefly radium, since most of the mesothorium had decayed to half strength by that time.

These so-called late cases of radiation osteitis were followed with startling results. The intense osteitis caused by years of irritative irradiation by the alpha particle had been transformed in a number of cases into sarcoma. Osteogenic sarcoma appeared in dial painters who had stopped work from six to eight to ten years previously. The sarcoma developed in the very cellular areas of replacement fibrosis.

I have now performed autopsies in four cases of osteogenic sarcoma among seventeen of the former dial painters whose death were due to the so-called radium

poisoning. This is an incidence of nearly 25 per cent. The sarcomas usually were rapidly growing and of the anaplastic or embryonal type. New bone formation was seen in all cases, but was often very slight. In one case there were two primary sarcomas, one in the orbit and another in the pelvis.

Further, three of the former dial painters with osteogenic sarcoma are now dying. The growths are in the femur, pelvis and rib. (Two of these were dead, June, 1931).

The development of sarcoma is suspected, but not yet proved, in three other dial painters.

This is the first time that definite proof is offered of minute amounts of radioactive substances causing cancer in the human body. This should not be confused with the development of epidermoid cancer, etc., in radium and x-ray burns produced by external penetrative, noncumulative radiation.

This is the first time that a definite cause for sarcoma of the bone is established aside from the time-worn and honored trauma, chronic irritation, etc.

The osteogenic sarcomas in the radium dial painters undoubtedly develop in areas that have previously been the seat of a radiation osteitis. In addition, one has also to consider the possibility that the alpha particle may have a more direct action in producing malignant growth by speeding up somatic cell division as a result of its destructive ionization.

It may be possible that the high incidence of carcinoma of the lung in the workers in the cobalt mines of Schneeberg (Saxony) and in the pitchblende mines of Joachimsthal, both radioactive mines, is also due to the irritant effects of radioactivity, the malignant tumors in the miners resulting from the constant inhalation of small amounts of emanation and also possibly radioactive dust.

It may be suggested that other forms of malignant growth are caused by still smaller amounts of radioactivity, working over longer periods of time, to which the human body, in its normal environment, is exposed, such amounts being too small to be recognized by present methods. These studies may also have an important part in the experimental production of malignant growth.

#### DISCUSSION

PAUL KLEMPERER: I was impressed by one histologic feature of the slides. Of course, I cannot add anything to the elaborate discussion by Dr. Martland, to whom we are all indebted for his discovery, but I noticed that nearly all the tumors of the bone showed one type of immature cell. He mentioned only one tumor in which there were numerous cartilage cells present, which he designated as a chondrosarcoma. In all the other tumors the prominent cell was a very immature cell which one could hardly classify among the mature types of bone cells. I do not know whether in these cases there is often bone or cartilage formation. If I am correct, it seems that all these tumors are characterized by a type of cell that we can call an immature mesenchymal cell, and since there is no question that these tumors arise in locations where radium is accumulated, it seems as though these tumors arise from a direct stimulation of the immature mesenchyme which persists throughout life in small amounts, but sufficient to take care of regeneration under normal conditions. If stimulated by some abnormal irritant, it may go on to proliferate and form tumors. I do not know if I understood Dr. Martland correctly, as to whether he thinks that the tumors are subsequent to the radium osteitis, that the osteitis is first produced by the action of radium, and then, as a kind of excess proliferation, a tumor is formed, or whether he would agree that the radium may act primarily on the mesenchyme to form tumors.

I would like to know whether there were any attempts made to determine the amount of radium in the bones that showed tumors and in those that did not show tumors, and if so, whether the bones with tumor showed more radium deposited than the other bones.

I should like to ask further whether experiments have been done to produce tumors in animals by the action of radium.

The point of my question is whether radium activates the growth of the mesenchyme. This is particularly interesting to me, because in my experience lymphosarcomas have changed their character biologically as well as histologically under persistent treatment by radiation. There were two cases reported by Baehr and Rosenthal, which were considered as representing a rather harmless disease of the lymph nodes, which responded very well under x-ray treatment, but which in the further course developed into lymphosarcomas composed of cells of a very immature type. A third case that it has been possible to follow for four and a half years has also shown this peculiar variation from the more mature type of lymphosarcoma into a young, immature type, which one would commonly call a reticulum cell lymphosarcoma. That is the reason why I am so interested in the question whether radium may activate the unripe mesenchyme, bringing it to proliferation, without giving the mesenchyme any chance to mature into one of the more ripe forms like fibroblasts.

That seems to me to hold also for the peculiar regenerative changes in the bone marrow. I was impressed by the fact that a number of the nuclei shown in the slides showed the peculiar pale appearance characteristic of the immature structures of the mesenchyme.

I was surprised to hear that Dr. Martland believes that radium might not only activate the mesenchymal structures, but also bring to neoplastic proliferation the epithelial cells, as in the tumors of Scheeberg. From his experience it seems that only the mesenchyme is activated to form tumors, but this might be due to the fact that the radium became deposited only in the bones. I wonder why in Saxony the radium should not become also deposited in the bones, and why there should not be a frequency of bone tumors; whether the radium is ingested or whether it gains entrance through the respiratory tract seems to me to make not a great deal of difference.

FREDERICK B. FLINN (by invitation): Speaking of the lesions of the skull bones, it seems rather strange that in Connecticut where there have been nineteen cases, twelve have shown these lesions. On autopsy it was found that the concentration of radium in these areas was not increased over that in other parts of the skull. It looks like a physical effect, in which the finer particles are broken off from the bone and then absorbed. In Connecticut there have been two cases in which spontaneous fractures of the femur occurred. I have been impressed by the fact that in the cases that have developed lately most of the lesions have occurred in the pelvic region, and these cases may sooner or later show the sarcoma of which Dr. Martland has spoken.

Speaking of animal experiments, rabbits have been treated with radium and mesothorium for a long period of time, and have shown lesions of the skull, as well as spontaneous fractures of the bones, but so far no sarcoma has developed—simply spontaneous fractures.

HARRISON S. MARTLAND (closing the discussion): Dr. Klemperer is quite right in his interpretation of the slides. The osteogenic sarcomas in the radium dial painters are usually of a very immature type. I have spoken of them as sarcomas of the embryonal or anaplastic type. In only one case have I encountered a mature sarcoma, in which good bone and cartilage had been laid down.

In addition the hyperplastic bone marrow seen in these cases is of a very immature and primitive type. Aside from the production of eosinophil myelocytes, the marrow has lost the ability to form other cells of the granulocyte series. The marrow is packed with the most primitive stem cells.

This irritative, stimulative and compensatory marrow is directly due to the terrific effects of alpha bombardment. A very cellular replacement fibrosis takes place. Mitotic figures are numerous, and such areas can only with great difficulty be distinguished from sarcoma. The sarcomas arise in these areas on a chronic irritative inflammatory basis. In addition, as Dr. Klemperer has suggested, we have to consider a more direct effect of radiation in speeding up somatic cell division. Perhaps both play a part.

We have been unable to determine that the bones containing the sarcomas had more radioactivity than did other bones of the skeleton. The studies seem to indicate that the radioactive substances are scattered rather evenly over the skeleton in much the same manner as lead or arsenic. Autophotographs made from the bones by means of their own radiations, however, show that the distribution is by no means even. I think this is on account of the marked architectural difference in many of the bones. Some have more cancellous tissue than others and would more easily pick up radioactive substances from the blood.

It would seem that experimental tumors could be produced in animals by internal alpha bombardment, if the conditions encountered in the dial painters could be duplicated. I believe that this is possible, although I have not as yet tried to produce tumors by means of radioactive substances.

If the pulmonary carcinomas in the miners of Schneeberg and Joachimsthal are due to radioactivity, they are probably produced by the inhalation of emanation or of radioactive dust or of both. If emanation is inhaled, the bronchi and alveoli would be directly exposed to alpha bombardment. Active deposit would also collect on the mucosa. Regarding radon, these deposits, although infinitely small, are sometimes long-lived, and would eventually reach the blood and be deposited in other organs. The amounts, however, would be so small that it is unlikely that bone sarcoma would ever develop.

## Book Reviews

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LEHRBUCH DER SPEZIELLEN PATHOLOGISCHEN ANATOMIE FÜR STUDIERENDE UND AERZTE. Band I. By DR. EDUARD KAUFMANN, ö. Professor der allgemeinen Pathologie und pathologischen Anatomie an der Universität Göttingen, Geheimer Medizinalrat. Ninth-tenth edition. Price, 55 marks. Pp. 990, with 509 illustrations. Berlin: W. de Gruyter and Company, 1931.

It is a delightful surprise to have this textbook, known for so many years as the "Bible of pathological anatomy," so completely rewritten. It is apparent that the task has been one of the chief interests of Professor Kaufmann since his retirement in 1928 from academic duties. There are many indications that the labor must have afforded him a great deal of sober enjoyment and satisfaction; for example, his continued painstaking interest in the use of numerous small expedients facilitating instruction: changes in type to attract attention, improved captions and subdivisions of information, and elaborate legends for sixty-eight added illustrations. The new illustrations conform to the old. They are simple and designed to teach. Among portions entirely new or extensively revised are those devoted to angina pectoris, blood grouping, allergic phenomena, eclampsia, esophageal spasm, endometriosis and sedimentation of erythrocytes. The author has thought proper to mention these changes in the preface, but there is scarcely a page without some embellishment, many being merely references to the recent literature. There are no long bibliographies, but instead, citation to places where the literature has been adequately reviewed. In this first volume alone there are more than 7,500 new citations, among them the names of many Americans. There probably would be more notice of both American and Canadian investigations if Professor Kaufmann's name was more generally included in reprint mailing lists. The brief anecdotes of personal observations have also been increased. Ever since the first edition appeared in 1896, medical literature has been permeated with citations to these case reports by Kaufmann. To supplement them once more, and also to obtain other material for this revision, he has ransacked the institutes where he formerly taught at Breslau, Basel and Göttingen.

Some of the descriptions of pulmonary tuberculosis, rheumatic myocarditis, endocarditis lenta, botulism and many diseases of the liver, bile ducts, gallbladder, thyroid and parathyroid glands have been so modernized that they are, so to say, up to the minute; others make their debut in an agreeable fashion and a propitious setting. Opportune and welcome are mild designations to apply to the accounts in different chapters of damage to organs by therapeutic radiation, for, unfortunately, as is too frequently the case, tragic clinical experiences with such treatments preceded animal experimentation. The rapid advance of knowledge of tumors during the period of nearly a decade since the last German edition was published has required many alterations in descriptions of neoplasms involving the organs and other important structures considered in this volume. Kaufmann has always been vigilant concerning tumors, a solicitude due possibly in some degree to the course on this subject he gave so many years for practitioners. Presumably expansion in these and innumerable other particulars brought about omission from this volume, of the final two chapters on diseases of bones and joints in volume 1 of the last edition. Even with this change, a liberal use of smaller type has been resorted to in order to retain the former size of this volume. It contains only four chapters, in which are presented the pathology of the organs of circulation; of the blood, lymph and blood-forming organs; of the respiratory organs, thymus, thyroid and parathyroid glands, and, in the final chapter, of the alimentary canal, peritoneum, liver, biliary apparatus and pancreas.

Approving or belauding this work to experienced pathologists is certainly uncalled for. Medical students, however, and the younger generation of investigators and teachers in pathology may perhaps be reminded of the even, high, informative value it has, that it is the outgrowth of a rich background of expe-

rience, the product of an able scholar who has exhibited unusual and critical intelligence in maintaining for the textbook the place it has enjoyed so long as an authority of the first rank. The reception given this ninth and tenth edition will certainly be followed by a widely echoed hope that the author may be privileged to supervise the preparation of many other future editions.

**RESISTANCE TO INFECTIOUS DISEASES: AN EXPOSITION OF THE BIOLOGICAL PHENOMENA UNDERLYING THE OCCURRENCE OF INFECTION AND THE RECOVERY OF THE ANIMAL BODY FROM INFECTIOUS DISEASE, WITH A CONSIDERATION OF THE PRINCIPLES UNDERLYING SPECIFIC DIAGNOSIS AND THERAPEUTIC MEASURES.** By HANS ZINSSER, M.D., Professor of Bacteriology and Immunity, Medical School, Harvard University; formerly Professor of Bacteriology at the College of Physicians and Surgeons, Columbia University, and Bacteriologist to the Presbyterian Hospital, New York; formerly Professor of Bacteriology and Immunity, Stanford University, California. Fourth edition, completely revised and reset. Price, \$7. Pp. 651. New York: The Macmillan Company, 1931.

This book, now in its fourth edition, "completely revised" and provided with a new name, appeared first in 1914 under the title of "Infection and Resistance." There are two sections: (1) general principles of infection and resistance and (2) special problems of immunology and consideration of individual infectious diseases. Since the edition published in 1924, the progress of research has changed the outlook in immunology in important respects. The advances in knowledge of the chemistry of antigens and of the mechanisms of antigen-antibody reactions receive due consideration in the present revision. One wonders why both in the text and in the index Landsteiner's term for that important factor, the partial antigen, is spelled "haptene" in some places and "haptine" in others. The alteration in antigenic properties of bacteria in the course of dissociation is considered in connection with certain infections, but any one who may wish to consult the book on that point will not receive help from the index, which contains no reference to dissociation or mutation. The interesting immunologic problems of tularemia and undulant fever do not appear to have received any consideration, and these diseases are not mentioned in the index. The suggestion that the toxins of erysipelas and of scarlet fever may be identical is not in accord with the results of the best recent investigations of that question.

On page 618 occurs this statement: "Force (1914) has described an immediate reaction after revaccination, which he believes occurs only in individuals in whom immunity exists. In such persons an areola occurs around the vaccinated spot within twenty-four hours which decreases within seventy-two hours." This reaction was observed and correctly interpreted by Jenner, described at great length by James Bryce in 1802, and rediscovered independently by Pirquet and no doubt by others also. The index could have been much better. The few illustrations are too crude to be of any value. Judging from the references, which do not follow any standard style, much more detailed consideration has been given to the earlier than to the more recent literature. This does not necessarily mean that recent contributions have been neglected, because it may indicate merely a different, more condensed form of presentation.

While the book suffers from omissions, from patchy and perhaps hasty revision and from other obvious shortcomings, the discussions of certain large problems in immunology are comprehensive and stimulating. This is illustrated especially in the chapters on anaphylaxis. Here the able author reveals his best strength.

**NOGUCHI.** By GUSTAV ECKSTEIN. Price, \$5. Pp. xiii and 419, with a portrait and several illustrations from photographs. New York: Harper and Brothers, 1931.

Good biographies are rare, good medical biographies almost unknown, wherefore the advice to read this one. To pathologists and bacteriologists who know

their fields there is no purpose in restating the facts of the life of Noguchi or his accomplishments. They will read this volume because it brings much more—the human side of a scientific worker, the story of his weaknesses and his strengths, the illustration in one man of what many are striving to be.

The public cherishes its physicians because it still believes them to be the best carriers of certain ideals and their lives romantic. Eckstein's biography will do much to fortify this faith. He puts in print the handicaps, the ambitions, the toil, the self-sacrifice of Noguchi and finishes the romance of his life with his death from yellow fever (there is no romance, as William James has it, without the possibility of sudden and violent death). Such a tale in the real of a contemporary worker in medicine will do much to reestablish in the public mind its old imagining of the profession's life.

Noguchi was born Japanese, lived American and worked internationally. The resultant emotional reactions are splendidly portrayed. Perfect adjustments to new environments were not made even by Noguchi, but what he accomplished in this direction was possible only in the cloak of medicine.

If it is said that Eckstein will live because he had a good subject, it is equally true that another great character in medicine would have faded from consciousness had it not been for Eckstein. The dates, incidents, honors, letters which constitute the ordinary mortuorial note cannot be strung together even as the newspaper men are wont to do and yield anything strong enough to hold together for a week. Eckstein uses these things, but they become the foundations of a soul afire. It could be done only by a man who knew Noguchi, his friends the world over, Japan in Japan and human drama no matter where played. As dentist, doctor, worker in the sciences basic to medicine, world traveler and, always, as writer, Eckstein brought to his task rare qualities. His volume exhibits them and would have been literature without its title.

The style is staccato. It fits peculiarly well a recitation of the ways of life and the ways of work of Noguchi and blends perfectly with the literal translations of the letters in the volume from the Japanese. Some will think that Eckstein adopted his style from this source, but a knowledge of his other writings proves it his own. The world of medicine will feel proud that Noguchi belonged to it and that his biographer is a part of it.

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Die MORPHOLOGIE DER MISSBILDUNGEN DES MENSCHEN UND DER TIERE. EIN HANDBUCH UND LEHRBUCH FÜR MORPHOLOGEN, PHYSIOLOGEN, PRAKTIISCHE AERZTE UND STUDIERENDE. HERAUSGEGEBEN VON DR. GEORG B. GRUBER, o. ö. Professor der Pathologie an der Universität Göttingen. Unter Mitwirkung zahlreicher Fachgenossen, begründet von Weil und Prof. Dr. Ernst Schwalbe. III. Teil: Die Einzelmissbildungen. XIV. Lieferung. 3. Abteilung. 5. Kapitel: Die Entwicklungsstörungen der Hypophyse. Von Prof. Dr. E. J. Kraus, Prague. With 21 illustrations in text. 6. Kapitel: Missbildungen der Nebennieren. Von Dr. W. Pagel, Sommerfeld-Osthavelland. With 20 illustrations in text. 7. Kapitel: Die Missbildungen der Epiphyse des Gehirns. Von Dr. H. Wurm, Heidelberg. With 5 illustrations in text. Jena: Gustav Fischer, 1929.

Previous parts of this great work dealing with the malformations of man and animals have been reviewed in the ARCHIVES (4:504, 1927; 8:571, 1929). The present section deals with developmental disturbances of the hypophysis, the suprarenal glands and the pineal body. An extensive bibliography is appended to each chapter. The numerous illustrations, some in colors, are excellent. The subject matter in the chapter on the suprarenal glands may be cited as indicating the scope of the work in general: comparative anatomy, developmental history, agenesis, hypoplasia, hyperplasia and congenital hypertrophy, misplacements and accessory and aberrant suprarenal glands. There are full data on the size and weight of these organs. The reviewer would call particular attention to the discussion of

accessory and aberrant suprarenal glands. As has been said of previous sections, this treatise is of the utmost value as a reference book for pathologists and anatomists.

EINFÜHRUNG IN DIE MEDIZIN. By DR. HENRY E. SIGERIST, Professor an der Universität Leipzig. Price, 12.50 marks. Pp. 405. Leipzig: Georg Thieme, 1931.

The author is Sudhoff's successor as professor of medical history in the University of Leipzig. He addresses himself especially to young persons about to begin the study of medicine. His object is to give them needed insight into the task ahead of them by means of a simple, yet comprehensive, conspectus of medicine as a whole on a historical or a developmental basis. There are seven chapters. The first deals with the structure and function—*anatomy, physiology and psychology*—of normal man. The necessity for the physician to acquire a broad knowledge of man in all his relationships is stressed. The next four chapters discuss the patient and disease. The growth of the concept of disease, its course and causes are discussed with admirable clearness. Then there is a chapter on diagnosis, healing and prevention. The last chapter is devoted to the doctor himself and his relation to society.

The book is written in a remarkably clear, direct and effective style. Typographically, the failure to indent the paragraphs may be confusing to the reader.

The book is highly interesting and will be of great help to medical students at the beginning of their course. It is recommended to all who are interested in the development of medicine and especially to medical educators. Sigerist shows clearly that as the developmental relations of various subjects in the study of medicine come to be understood, the sense of discontinuity in the medical curriculum, especially in the first two or three years, tends to vanish. The book merits translation into English.

WILLIAM STEWART HALSTED, SURGEON. By W. G. MACCALLUM. Introduction by DR. W. H. WELCH. Price, \$2.75. Pp. 246. Baltimore: Johns Hopkins Press, 1931.

This book may be accepted as an authoritative biography of William S. Halsted, the first professor of surgery in the Johns Hopkins University. In his characteristically admirable introduction, Dr. William H. Welch, who was Dr. Halsted's closest friend and probably knew him better than any one else, explains in detail why he can give the book his unqualified approval. The book gives an interesting and adequate account of Halsted's great achievements as teacher, surgeon and experimental investigator. It may be recalled that he was the first to produce local anesthesia by nerve blocking; that he developed an improved surgical technic aimed to secure a more ideal mode of healing; that rubber gloves were used first in his clinic; that his operations for cancer of the breast and for hernia established new records of success in their respective fields; that he conducted highly important experimental investigations, notably on the thyroid gland and on intestinal suture, and, finally, that he developed a school of surgeons that so far is without parallel in this country. The account of these and other achievements in MacCallum's book reproduces successfully original settings and is tinged with pleasant local colors. One is not confronted with a lifeless plaster image of Halsted. The outward facts of his life, strength of character, ideals and peculiarities are set forth clearly and vividly. An atmosphere of intimate certainty, of affectionate loyalty with complete candor prevails. The book is good reading. It is a noteworthy addition to medical biography.



## Books Received

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DIE EPIDEMISCHE KINDERLÄHMUNG. Von Prof. C. W. Jungeblut, Oberregierungsrat Dr. E. Roesle, Prof. Levaditi, Privatdozent de Rudder, Privatdozent A. Lichtenstein, Oberarzt Dr. P. Bamberger, Dr. R. W. Fairbrother, Prof. H. Schlossberger und Prof. P. Pitzen. Price, unbound, 8 marks; bound, 10 marks. Pp. 132. Munich: Verlag der arztlichen Rundschau Otto Gmelin, 1931.

STUDIES OF PROTECTION AGAINST TUBERCULOSIS: RESULTS WITH BCG VACCINE IN MONKEYS. By A. Stanley Griffith. Medical Research Council, Special Report Series no. 152. Price, 9 pence, net. Pp. 49. London: His Majesty's Stationery Office, 1931.

MEDICINA FENNICA VI ANNO MCMXXX. Edidit Societas Medicorum Fennica Duodecim. Pp. 171. Helsinki: 1931.

MEDICAL JURISPRUDENCE. By Alfred W. Herzog, Ph.B., A.M., M.D., Honorary Academician of the International Academy of Letters and Sciences. Price, \$15, cloth. Pp. 1051. Indianapolis: Bobbs-Merrill Company, 1931.

LA CULTURE DES TISSUS EN BIOLOGIE EXPÉRIMENTALE. Par Emile C. Cracium, Maître de Conférences à la Faculté de Médecine de Bucarest; Préface du Prof. G. Roussy. Price, 55 francs. Pp. 442, with 72 illustrations. Paris: Masson et Cie, 1931.

PHYSIOPATHOLOGIE DE LA THYROÏDE DIAGNOSTIC ET TRAITEMENT DES GOITRES. Par Lucien Dautrebande, de la Fondation Reine Elisabeth; Membre correspondant de l'Académie royale de Belgique. Price, 40 francs. Pp. 326, with 36 illustrations. Paris: Masson et Cie, 1931.

MYOCARDIUM IN YELLOW FEVER. University of Toronto Studies: Pathological Series, no. 8. By Wray Lloyd, Banting Fellow in the Department of Pathology. Pp. 172. Toronto: University of Toronto Press, 1931.

METHODS AND PROBLEMS OF MEDICAL EDUCATION (Nineteenth Series). Medicine in the Division of Biological Sciences, University of Chicago. New York: The Rockefeller Foundation, 1931.

## PERIARTERITIS NODOSA COMPLICATED BY FATAL INTRAPERICARDIAL HEMORRHAGE

REPORT OF A CASE \*

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AND

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NEW YORK

Periarteritis nodosa or polyarteritis nodosa is a more or less generalized inflammation of the smaller arteries characterized at the beginning by acute degenerative and reactive changes in the vascular walls and later by the development of thrombosis, aneurysms and sclerosis. Clinically the symptomatology is protean, and a correct diagnosis during life is difficult. The prognosis is unfavorable; most of the patients die within a few months of the appearance of the symptoms. They either succumb to the toxemia resulting from extensive degenerative changes in the different organs and tissues or collapse suddenly from a fatal hemorrhage due to rupture of a small aneurysm.

Since the first comprehensive description of periarteritis nodosa by Kussmaul and Maier<sup>1</sup> in 1866, about 150 cases in man have been described in the literature. The ages of the patients varied from 3 months to 78 years, though the majority were in the third and fourth decades of life. Males were affected about four times as frequently as females (Arkin<sup>2</sup>). In addition, investigators have recorded cases of the condition in deer, cattle, swine and dogs (Nieberle<sup>3</sup>).

The etiology has been the subject of much speculation. Some writers (Ferrari,<sup>4</sup> Benda<sup>5</sup> and Meyer,<sup>6</sup> for example) have held that the underlying cause was weakness of the vascular walls induced by various toxic influences. Eppinger<sup>7</sup> referred all the lesions to congenital deficiency of the arterial media. Other writers (Graf,<sup>8</sup> Schmorl<sup>9</sup> and Verse<sup>10</sup>)

\* Submitted for publication, March 16, 1931.

\* From the Pathological Laboratory and the First Medical and Surgical (Columbia) Divisions of Bellevue Hospital.

1. Kussmaul, A., and Maier, R.: *Arch. f. klin. Med.* **1**:484, 1866.
2. Arkin, A.: *Am. J. Path.* **6**:401, 1930.
3. Nieberle: *Virchows Arch. f. path. Anat.* **256**:131, 1925.
4. Ferrari, E.: *Beitr. z. path. Anat. u. z. allg. Path.* **34**:350, 1903.
5. Benda, C.: *Berl. klin. Wchnschr.* **45**:353, 1908.
6. Meyer, P.: *Virchows Arch. f. path. Anat.* **74**:277, 1878.
7. Eppinger, H.: *Arch. f. klin. Chir. (supp.)* **35**:42, 1887.
8. Graf, E.: *Beitr. z. path. Anat. u. z. allg. Path.* **19**:181, 1896.
9. Schmorl: *Verhandl. d. deutsch. path. Gesellsch.* **6**:203, 1904.
10. Verse, M.: *Beitr. z. path. Anat. u. z. allg. Path.* **40**:409, 1907.

considered the disease to be a form of syphilitic aortitis. Aschoff<sup>11</sup> suggested rheumatic infection as a possible causative factor. Many investigators (Klotz,<sup>12</sup> Lamb,<sup>13</sup> Jonas<sup>14</sup> and Ophüls<sup>15</sup>) have isolated different strains of pathogenic bacteria from cases of periarteritis nodosa, but such organisms have not filled adequately the rôle of an etiologic agent. All these theories have been found unsatisfactory and have been abandoned.

At present, opinion is sharply divided between the theory of von Hann,<sup>16</sup> who claimed that periarteritis nodosa is a disease entity caused by the action of a specific filtrable virus, and the theory of Spiro<sup>17</sup> and Gruber,<sup>18</sup> who claimed that the lesions are referable to the actions of many different noxious substances. Gruber is of the opinion that the arterial inflammation is a cellular reaction of the hyperergic type elicited by some toxin to which the tissues are sensitive. Unfortunately it is not possible to decide definitely at present on the correctness of these theories, as either one offers an equally good explanation of the pathologic changes encountered.

The results obtained by experimental inoculation of animals have been contradictory. Harris and Friedrichs<sup>19</sup> claimed that they had produced the disease in rabbits by inoculation with material from a case in man. Von Hann<sup>16</sup> asserted that he had in a similar fashion infected guinea-pigs. On the other hand, the experiments of Kopp,<sup>20</sup> Lemke,<sup>21</sup> Carling and Hicks,<sup>22</sup> Ophüls,<sup>15</sup> Sacki,<sup>23</sup> Otani<sup>24</sup> and Klotz<sup>12</sup> showed negative results. Evidently the final word on the etiology of this condition must be left to the future.

The arterial lesion in periarteritis nodosa may be characterized as primary necrosis of the wall followed by an attempt on the part of the body to repair the damage. The series of changes that occur may be described most effectively by dividing the process into four stages, as recommended by Arkin.<sup>2</sup> It must be kept in mind, however, that this

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11. Aschoff: *Verhandl. d. deutsch. path. Gesellsch.* **10**:157, 1906.

12. Klotz, O.: *J. M. Research* **37**:1, 1917.

13. Lamb, A. R.: *Arch. Int. Med.* **14**:481, 1914.

14. Jonas: *München. med. Wchnschr.* **59**:1685, 1912.

15. Ophüls, W.: *Arch. Int. Med.* **32**:870, 1923.

16. von Hann, F.: *Virchows Arch. f. path. Anat.* **227**:1, 1919.

17. Spiro, P.: *Virchows Arch. f. path. Anat.* **227**:1, 1919.

18. Gruber, G. B.: *Virchows Arch. f. path. Anat.* **258**:441, 1925.

19. Harris, W. H., and Friedrichs, A. V.: *J. Exper. Med.* **36**:219, 1922.

20. Kopp, G.: *Deutsche med. Wchnschr.* **49**:1239, 1923.

21. Lemke, R.: *Virchows Arch. f. path. Anat.* **245**:322, 1923.

22. Carling, E. R., and Hicks, J. A. B.: *Lancet* **1**:1001, 1923.

23. Sacki, F.: *Med. Klin.* **20**:45, 1924.

24. Otani, S.: *Frankfurt. Ztschr. f. Path.* **30**:208, 1924.

division is more or less artificial, and that in a particular case the different stages may not be separated from each other in a clearcut fashion. The four stages are: (1) the primary or degenerative stage, (2) the acute inflammatory stage, (3) the granulation tissue stage and (4) the scar tissue or healed stage.

1. The primary stage is ushered in by acute coagulation necrosis of the media of the smaller arteries. The tissue immediately adjacent is filled with edematous fluid, and fibrin is deposited in the spaces. Polymorphonuclear leukocytes and a few eosinophils invade the affected area. The intimal endothelium becomes more permeable than in normal conditions. It may be crowded into the lumen of the blood vessel by the exudate in the subintimal space. In this early stage the adventitia is, as a rule, not involved.

There has been much discussion in the literature concerning which portion of the arterial wall is inflamed the earliest. As Gruber pointed out, however, the provocative agent reaches the vessel through the vasa vasorum, and the greatest reaction occurs in the layer of the wall that contains the most profuse supply of capillaries. In the larger vessels the outer layer of the media suffers the earliest, and in the smaller vessels the inner layer of the media is the first to be involved, because at these respective points the capillaries are the most abundant. In the arterioles, which lack vasa vasorum, the subendothelial layer shows the characteristic lesion, apparently because the causative agent attacks the vessel from the lumen. The location of the primary area of necrosis seems to depend on the size of the affected vessel.

The degree of arterial involvement is variable in different cases. In some instances only a portion of the circumference may be affected; in others a large annular segment may be inflamed. The number of arteries attacked also varies within wide limits. In case 1 described by Arkin,<sup>2</sup> there was a periarteritis nodosa throughout the body, while in the case reported by Fishberg<sup>25</sup> the arteries of the kidneys alone were affected. As a rule, however, most examples of the disease fall between these two extremes.

It has been estimated by Gruber (cited by Carr<sup>26</sup>) that the vessels are involved in the following relative frequency: the renal arteries, 80 per cent; the coronary arteries, 70 per cent; the hepatic arteries, 65 per cent; the arteries of the gastro-intestinal tract, 50 per cent; the pancreatic arteries, 25 per cent; the mesenteric arteries, 30 per cent; the arteries of the muscles, 30 per cent; those of the peripheral nerves, 20 per cent, and those of the brain, 8 per cent.

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25. Fishberg, A. M.: *Virchows Arch. f. path. Anat.* **240**:483, 1923.

26. Carr, J. G.: *M. Clin. North America* **13**:1121, 1930.

2. In the acute inflammatory stage the media becomes completely necrotic, and the elastic tissue is destroyed. The vascular wall is infiltrated throughout by polymorphonuclear leukocytes, eosinophils, lymphocytes and plasma cells. The process may extend to the surrounding tissues so that an inflammatory perivascular zone forms around the vessel. The inflammation is acute, but never acquires frankly suppurative characteristics. The adjacent veins may be included in the exudate because of their proximity, but rarely show any lesions comparable with the process in the arteries. Walter<sup>27</sup> and Kountz,<sup>28</sup> however, described a mesophlebitis in their cases.

3. Sooner or later the polymorphonuclear leukocytes gradually disappear and are replaced by eosinophils, lymphocytes and plasma cells. At the same time granulation tissue wanders in from the adventitia, pervades the entire thickness of the vascular wall and even extends into the perivascular zone. The type of lesion at this stage indicates that the acute process has begun to subside and that repair is in progress.

During the progress of stages 2 and 3, the process in the arterial wall shows a tendency to develop in two different ways. In some vessels, the subendothelial connective tissue is incited to proliferate by necrosis of the media. This encroaches on the lumen of the vessel and narrows it eccentrically or concentrically; in a few instances the artery may be obliterated either by the intimal lesion or by the formation of secondary thrombi. In other arteries, the wall is weakened by the inflammation and small aneurysmal dilatations are formed along the course of the vessel. Thrombi occur in such conditions. Ruptures of the aneurysms are common, and blood may be extravasated into the surrounding tissues. Occasionally the hemorrhage may take place in one of the large cavities of the body and be fatal.

Generally the obliterating type of endarteritis and the small aneurysmal dilatations occur in the same case, though the latter tend to predominate. However, in some instances, aneurysms may be absent, and the arterial inflammation can be demonstrated only by microscopic examination (Wohlwill<sup>29</sup>).

At necropsy the aneurysms may be found in any part of the body, but are observed characteristically as nodules, measuring from 2 to 20 mm. in diameter, along the course of the coronary arteries, just under the pericardium, or as subperitoneal nodules along the insertions of the mesenteries to the various parts of the gastro-intestinal tract. They have been noticed in the peripheral nerves, in voluntary muscles, in the subcutaneous tissues and in the submucous layer of the stomach or of the intestines. In the solid viscera, as the liver and the kidneys, they

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27. Walter, H.: *Frankfurt. Ztschr. f. Path.* **25**:306, 1921.

28. Kountz, W. B.: *Arch. Path.* **10**:55, 1930.

29. Wohlwill, F.: *Virchows Arch. f. path. Anat.* **246**:377, 1923.

are found in the connective tissue framework of the organ. Most of them are filled with thrombi of the bland type.

The usual result of the arterial changes, whether the process is the obliterating endarteritis or the aneurysmal dilatation, is to obstruct the vessel and hinder the supply of blood to the various viscera, so that areas of degeneration occur. As a consequence, multiple, bland, wedge-shaped infarcts occur in the liver and kidneys. Less often corresponding changes occur in the spleen, lung, heart, brain, gastro-intestinal tract, muscles, nerves, testicles and skin. In a few instances smaller areas of degeneration, not obviously connected with the obstruction of the arteries, are seen and are attributed by some investigators to the direct action of the toxic agent on the tissues.

The symptoms described in cases of periarteritis nodosa are referable to the toxic action of the causative agent and to the pathologic lesions in the arteries and viscera. In the first stage the disease may be latent or at most disclosed by a rise of temperature. During the second stage, when the arteries are inflamed, the signs of an acute infection make their appearance, such as continuous or intermittent fever, chills, polymorphonuclear leukocytosis and secondary anemia. Enlargement of the lymph nodes and of the spleen may occur.

When the second stage becomes well developed and the various organs are affected in different degrees, the symptoms become diverse. In some instances the predominant manifestations are those of renal insufficiency. In others the signs are those of circulatory failure. In still others there is great abdominal pain with severe attacks of jaundice, diarrhea, vomiting and the like. A fourth type is marked by the appearance of muscular pains and paralyses and, in general, all the stigmas of peripheral neuritis and myositis. Occasionally all of these different symptoms may be combined in the same patient.

The clinical picture as a rule is so variable that the diagnosis during life is often impossible. Some writers believe that different combinations of signs are suggestive of periarteritis nodosa. Meyer,<sup>30</sup> for example, favored the association of chlorotic marasmus, polyneuritis or polymyositis and severe gastro-intestinal symptoms as a characteristic syndrome. However, the disease can be identified during life only if some of the affected tissue is excised and subjected to microscopic examination. The diagnosis was made in the cases reported by von Hann,<sup>16</sup> Benedict,<sup>31</sup> Kopp<sup>20</sup> and Schmorl<sup>9</sup> through the removal of a subcutaneous nodule, and in the case reported by Manges and Baehr<sup>32</sup> by the excision of an abdominal nodule during the course of a laparotomy.

30. Meyer, P. S.: *Berl. klin. Wchnschr.* **58**:483, 1921.

31. Benedict: *Ztschr. f. klin. Med.* **64**:405, 1907.

32. Manges, M., and Baehr, G.: *Am. J. M. Sc.* **162**:162, 1921.

In general, the prognosis of periarteritis nodosa is unfavorable and most of the patients die at some point during the second and third stages. Gruber<sup>18</sup> places the average length of life at from seven to nine months. Some patients have lived longer; the patient whose case was reported by Wohlwill<sup>29</sup> lived twelve months, and the one whose case was reported by Spiro<sup>17</sup> lasted one and a half years. Fishberg's<sup>25</sup> patient had a clinical history of six days, but the disease must have had a much longer period of latency. The typical course is for the patient to have periods of acute illness lasting a few days or weeks, alternating with periods of quiescence. The inference is that during the stage of exacerbation the inflammation is spreading to other arteries. This is confirmed by the histologic examination of material recovered at necropsy. It is not uncommon to find some arteries showing acute necrotic inflammation and others showing well developed chronic end-arteritis obliterans.

Death may result from the progressive cachexia which is referable to the inflammatory changes in the arteries and the degenerative changes in the viscera, or it may follow a sudden profuse hemorrhage from one of the smaller aneurysms in some part of the body. Such hemorrhages have been described as occurring in the brain (Dickson,<sup>33</sup> Arkin<sup>2</sup>), in the lung (Sternberg<sup>34</sup>), in the intestine (Lowenberg<sup>35</sup>), in the abdomen (Klotz,<sup>12</sup> Lemke<sup>36</sup>) and in the kidney (Schmidt,<sup>37</sup> Harris and Friedrichs,<sup>19</sup> Mertens,<sup>38</sup> Walter,<sup>27</sup> Fishberg<sup>25</sup>).

4. In some cases of periarteritis nodosa only a few arteries may be affected, and the process may not damage the important organs in a serious fashion. The acute inflammation then may subside, and the fourth stage or the stage of healing supervene. There is replacement of the injured vascular wall by scar tissue. This replacement occurs in three ways (Arkin<sup>2</sup>): One is by the continued proliferation of the subintimal connective tissue and the new formation of elastic fibrils. The second is by the organization and canalization of thrombi. The third is by the formation of scar tissue in and around the artery, forming typical nodular perivascular mantles. These processes tend to produce closure of the blood vessels, and degenerative changes and fibroses develop in the kidneys, liver, myocardium and other viscera. The patient who has reached this stage may live for years or until the reserve capacity of the damaged organs fails.

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34. Sternberg, C.: *Wien. klin. Wchnschr.* **38**:729, 1925.

35. Lowenberg, W.: *Med. Klin.* **19**:207, 1923.

36. Lemke, R.: *Virchows Arch. f. path. Anat.* **240**:30, 1923.

37. Schmidt, J. E.: *Beitr. z. path. Anat. u. z. allg. Path.* **43**:455, 1908.

38. Mertens, E.: *Klin. Wchnschr.* **1**:1841, 1922.

## REPORT OF CASE

The case of periarteritis nodosa reported here was that of a patient who died of an unusual complication.

A young Negro, aged 21, a plumber, was admitted to the First Medical Division of Bellevue Hospital, complaining of a severe pain in the midabdomen. He claimed that this pain started two weeks prior to admission, had been practically continuous since that time, and was most intense at night. It was so troublesome that he was afraid to eat for fear that the symptoms would increase. He had had one attack of vomiting about four or five days prior to admission.

On admission, physical examination disclosed a well developed and well nourished young man, rather somnolent in appearance, with throat and tonsils moderately inflamed. The abdomen was tender, especially on the right side. The temperature was 102 F. The rest of the examination disclosed nothing of importance. A tentative diagnosis of acute pharyngitis, acute gastro-enteritis and lead poisoning was made.

After four days' observation, the combination of symptoms led to the suspicion that there was an acute inflammatory process in the abdomen, such as cholecystitis or appendicitis. The patient was transferred to the wards of the First Surgical Division of Bellevue Hospital and an exploratory laparotomy was performed. At operation the peritoneal cavity was found to be normal. The abdominal wound was closed without further procedure and subsequently healed without complications.

The patient's symptoms, however, were not alleviated. The temperature continued high, and on the ninth day after the operation there was a recurrence of the abdominal pain. In addition, the leukocyte count revealed 27,400 cells, of which 90 per cent were polymorphonuclear leukocytes, 8 per cent lymphocytes, 1 per cent transitional cells and 1 per cent eosinophils. On the following day he had two convulsive seizures one hour apart. The tenderness on the right side of the abdomen was increased.

On the twenty-sixth day after operation, the patient was returned to the medical ward in about the same condition. Two days later, he suddenly died in the midst of a general tonic convulsion with a cry and biting of the tongue. The length of the clinical course since the onset of symptoms was forty-six days.

A blood culture taken on the sixteenth day after operation was negative. The Wassermann reaction of the blood was strongly positive (4 plus); that of the spinal fluid was negative. Examination of the urine two days before death showed only a sediment of pus and epithelial cells; fluoroscopic examination disclosed a slightly enlarged cardiac and aortic shadow. The other tests did not show anything unusual.

A positive diagnosis was not made, but it was believed that the patient was suffering from a generalized infectious process of unknown origin.

*Necropsy.*—Necropsy was performed four days after death. The body was that of a young Negro, 5 feet, 10 inches in height, weighing about 135 pounds (61.2 Kg.). The frame was slender; the muscular development was fair, and the nutrition was poor. There was a recently healed scar from an operation in the upper midline of the abdomen, about 5 inches (12.7 cm.) in length. No other abnormalities were noted externally.

On section, the abdomen showed some old, cobweb-like adhesions between the liver and the diaphragm and hyperplasia of the lymph nodes around the pancreas. The entire gastro-intestinal tract appeared normal on gross examination.



The liver was large, weighing 2,200 Gm. Multiple wedge-shaped anemic infarcts were scattered through the organ, especially under the capsule. They averaged from 4 cm. to 7 cm. in diameter and were yellowish. Some of the larger ones showed softening in the center, and the capsule immediately adjacent was thickened and covered with a rough, fibrinous deposit. The capsule of Glisson was increased in thickness. Some of the hepatic arteries were filled with grayish-red thrombi (fig. 1).

The spleen weighed 200 Gm and was normal in size and appearance, except for the presence of small grayish-yellow infarcts, measuring from 3 to 6 mm. in diameter, scattered here and there just under the capsule.

The pancreas was normal, but the pancreatic artery showed small aneurysmal dilatations (from 6 to 10 mm. in diameter) that were filled with grayish-red thrombi.



Fig. 1.—Segment of liver showing multiple infarcts.

The suprarenal glands were normal.

The kidneys together weighed 600 Gm. They were dull purplish brown, with a smooth outer surface. They were permeated with wedge-shaped anemic infarcts, from 3 cm. to 10 cm. in diameter, which were grayish yellow and situated just under the capsule. The vessels at the hilus of the kidneys were distended and filled with grayish-red thrombi.

The bladder, prostate, testes, aorta, cervical organs, brain and lungs were normal in appearance. The peripheral nerves and the skeletal muscles were not examined.

The bronchial lymph nodes were enlarged and matted together, and showed caseous tuberculosis. It is probable that these nodes played a part in increasing the size of the aortic shadow in the fluoroscopic examination.

The pericardial cavity was distended, and the heart was compressed by 300 Gm. of fluid blood and a blood clot. When the clot was removed, it was found to be attached to a bright red subepicardial nodule, 8 mm. in diameter, adjacent to the

descending branch of the left coronary artery, about 4 cm. distant from the aorta. This was found to be a small aneurysm of the coronary artery which had evidently ruptured, causing a fatal hemorrhage into the pericardial cavity. Unfortunately, the opening found in the nodule could not be identified with certainty as an ante-mortem rupture. Numerous other subepicardial aneurysms, from 5 to 20 mm. in diameter, were present, especially on the anterior surface of the heart, coming off from small branches of the coronary arteries, but not involving the main trunks. Section of the aneurysms disclosed the presence of grayish-red thrombi. They



Fig. 2.—Heart showing aneurysms of smaller branches of coronary arteries.

gave the heart a lumpy appearance. The heart itself weighed 340 Gm. and was markedly contracted. It did not show anything else unusual (fig. 2).

*Diagnosis.*—The diagnosis made at necropsy was: periarteritis nodosa involving the arteries of the heart, liver, kidneys, spleen and pancreas; rupture of a small branch of the left coronary artery; hemorrhage into the pericardial cavity with compression of the heart; multiple infarcts of the liver, kidneys and spleen, and tuberculosis of the bronchial lymph nodes.

*Histologic Observations.*—The various tissues were fixed in formaldehyde and embedded in celloidin, and sections were stained in hematoxylin and eosin, as a routine. A few sections were stained with van Gieson's stain combined with

Weigert's elastic tissue stain. A few were stained with Levaditi silver stain for spirochetes, but these organisms could not be found.

The infarcts in the liver and kidney were demonstrated, on section, to be the usual type of anemic infarct.

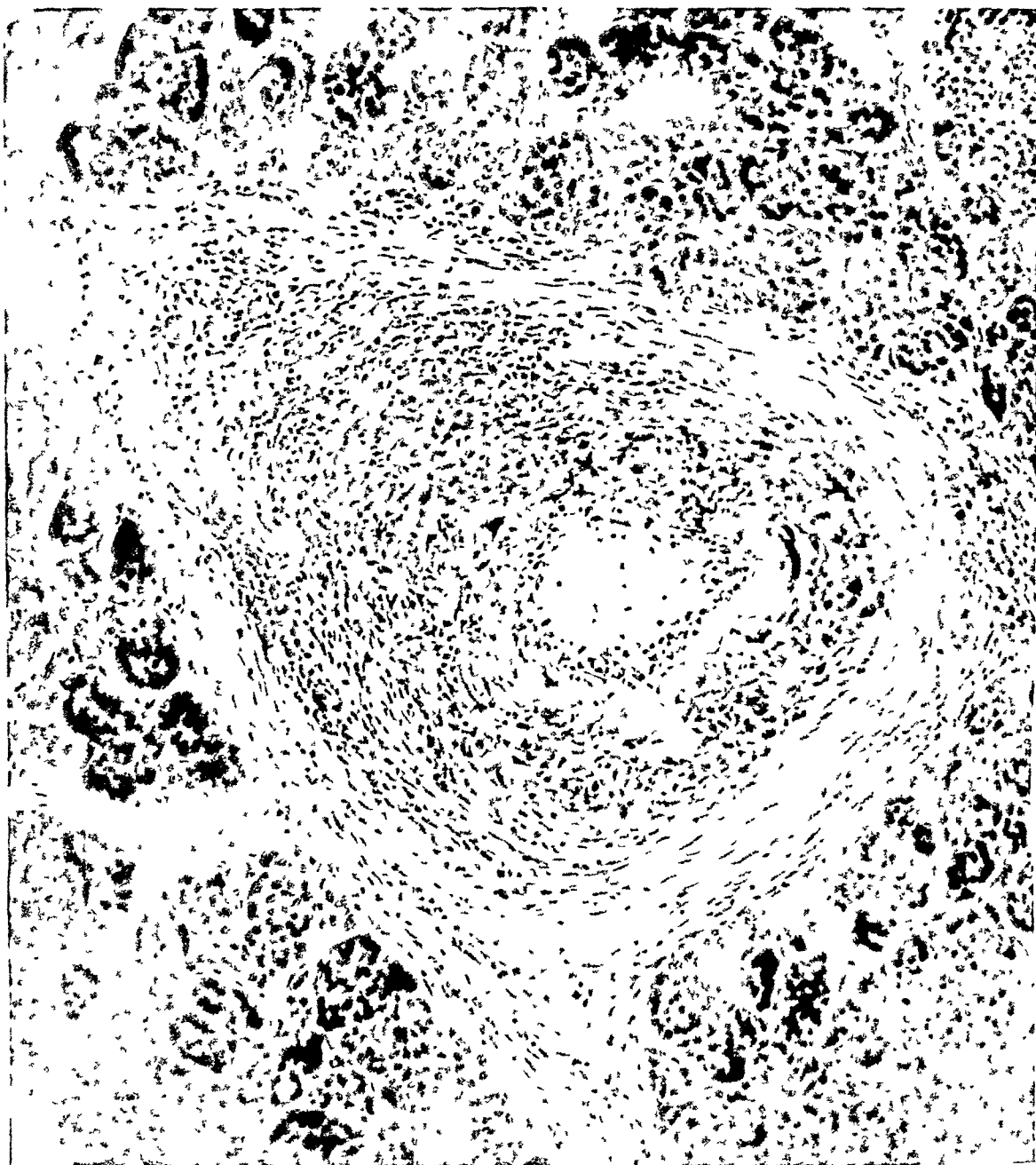


Fig. 3.—Small artery of pancreas in stage of acute inflammation: media necrotic and all layers infiltrated with leukocytes and other cells.

The caseous areas in the bronchial lymph nodes were found to represent a chronic tuberculous process. Sections through the lymph nodes around the pancreas showed merely lymphoid hyperplasia, probably the result of irritation.

The heart, liver, kidneys, pancreas, stomach, duodenum, mediastinum and epididymis were the seat of arterial inflammation. In other tissues, such as the aorta, lungs, central nervous system, skin and lymph nodes, lesions of this type were not found.

The histologic picture presented by the inflamed arteries was typically that of periarteritis nodosa. Early lesions characteristic of stage 2 were found in the stomach and duodenum and could be demonstrated only on microscopic section. The affected arteries were located in the subperitoneal layer and the outer muscular layer, while the arteries in the mesentery and submucous layer were normal. The media of the inflamed vessel was swollen, fibrillar, necrotic and markedly eosinophil. It was infiltrated with polymorphonuclear leukocytes and with a smaller portion of eosinophils, lymphocytes and plasma cells. The adventitia showed the same type of exudate and, in addition, numerous large ovoid cells were present; this layer was widened to several times the normal thickness. The subintimal layer was infiltrated with polymorphonuclear leukocytes, and the endothelium was pushed into the lumen by the process. The elastic tissue in the artery was destroyed. Some of the arteries of the mediastinum, epididymis and pancreatic tissues showed the same process (fig. 3).

In addition, an endarteritis obliterans suggesting the latter part of stage 3 was present in many small arteries of the epididymis, pancreas, heart, liver and kidneys. The lumen of the artery was narrowed and in some cases practically occluded by the proliferation of the fibrous tissue of the intima. The musculature was intact, and the elastic fibers were fairly well developed as a rule. The adventitia was normal, except in a few instances where there was a considerable deposit of brownish, granular, intracellular pigment.

The aneurysms in the heart, liver and kidneys were large vessels containing bland, laminated thrombi. The cells of the muscular layer were fairly well preserved, but areas of necrosis were found in the media here and there. The media and the adventitia were both extensively infiltrated with granulation tissue, polymorphonuclear leukocytes and other inflammatory cells.

The veins in the different sections were not inflamed.

In the heart and kidneys small collections of leukocytes were found without any apparent connection with the lesions in the blood vessels. In the tissues of the posterior mediastinum, many areas of infiltration by plasma cells were noted. It is possible that these lesions were caused by the toxemia.

The spleen and one of the pericardial aneurysms were cultivated for bacteria, but the results were negative.

#### SUMMARY

An example of periarteritis nodosa in a young colored man, 21 years of age, is depicted. The chief symptoms were severe abdominal pain, polymorphonuclear leukocytosis and a temperature of 102 F. A laparotomy was performed under the impression that the patient was suffering from an acute abdominal condition, but the peritoneal cavity was found to be normal. Subsequently the patient's condition did not improve, and forty-six days after the onset of symptoms he died in the midst of a generalized convulsion.

Necropsy disclosed multiple aneurysms of the coronary arteries and a hemorrhage into the pericardial cavity from the rupture of one of them. Aneurysms also were found in the arteries of the pancreas, liver,

kidneys and spleen. An acute necrotic arteritis and a chronic obliterating arteritis without the formation of aneurysms were noted in the pancreas, mediastinum, epididymis and gastro-intestinal tract. Multiple infarcts were present in the liver, kidneys and spleen. The pathologic changes were typical of periarteritis nodosa and were sufficient to account for the clinical symptoms of the patient.

The length of the clinical course was forty-six days—an apparently rapid course—but there is reason for believing that the arterial inflammation began long before the symptoms started. In some places the patient showed an acute necrotic arteritis of recent origin and in other areas an endarteritis obliterans of much longer duration. Evidently the different vessels were affected at different intervals.

# EFFECTS OF ULTRAVIOLET RAYS, RADIUM AND X-RAYS ON PROTEINS \*

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The increasing use of ultraviolet rays, radium and x-rays as therapeutic agents has awakened the interest of the scientific world in the changes that these same agents produce in the substances of the organism. This is especially true with respect to what is probably the most important constituent of protoplasm, protein.

The changes occurring in irradiated solutions of proteins may be considered from both the physicochemical and the purely chemical points of view. Of these, the physicochemical point of view has proved especially satisfactory and fruitful when applied to colloids in general and to proteins in particular, as in the well known investigations of Hardy, Robertson and Pauli. The following studies on the effects of irradiation with rays of short wavelength on various types of protein solutions were made, therefore, with the aid chiefly of physicochemical methods.

1. The effects of irradiation on a solution of protein depend on whether or not the particular protein is soluble in water and whether the solution contains an electrolyte or is electrolyte-free. Neglect of these factors probably accounts for the discordant results recorded in the literature. Only water-soluble proteins can, of course, be studied in an electrolyte-free condition. Irradiation of electrolyte-free solutions of serum albumin or of pseudoglobulin results in a denaturation and coagulation of the protein, as was first shown by Hardy<sup>1</sup> for radium, by Dreyer and Hanssen<sup>2</sup> for ultraviolet rays and by myself<sup>3</sup> for x-rays. In 1 per cent solution of albumin, the first sign of flocculation appears after ten minutes' irradiation at a distance of 30 inches

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\* From the Department of Colloid Chemistry, Temple University Medical School, D. T. McCarthy Foundation, Philadelphia.

\* The experimental work referred to in this paper was partly carried out in the Institutes for Physiology and Medical Colloid Chemistry of the University of Vienna, Austria.

\* The author is indebted to Dr. Arthur G. Cole, of the University of Illinois, College of Medicine, for aid in the preparation of the English version of this paper.

1. Hardy, W. B.: *J. Physiol.* **29**:29, 1903.

2. Dreyer, G., and Hanssen, O.: *Compt. rend. Soc. de biol.* **145**:234, 1907.

3. Spiegel-Adolf, M.: Unpublished observations.

(76.2 cm.) from the mercury arc,<sup>4</sup> after nine hours' irradiation with 80 mg. of metallic radium<sup>5</sup> or after exposure to at least 72 Holzknacht units of x-rays.<sup>3</sup> These data were obtained with sterile solutions of protein, since Spiegel-Adolf and Pollaczek<sup>6</sup> showed that bacterial infection affects the time required and may even entirely prevent the flocculation of the protein. If, however, irradiation is continued on sterile solutions, precipitation of the protein results. It is necessary to point out, in connection with these experiments, that the electrolyte-free solutions of protein need not be at their iso-electric points in order to achieve these results.

2. There is little information available concerning the nature of the chemical changes that proteins undergo during denaturation. The investigations of Harris<sup>7</sup> indicated that denaturation is accompanied by oxidation. The amount of oxygen bound by the protein must, however, be small, since coagulation by ultraviolet rays is observed in an atmosphere of nitrogen that contains only small amounts of oxygen.<sup>8</sup> Experiments made by Spiegel-Adolf and Fernau<sup>9</sup> indicated that the nitrogen content of proteins is not changed by coagulation with radium. These results suggest the possibility that denaturation of proteins by various types of rays involves a physicochemical rather than a chemical change in the protein.

3. In order to analyze the character of the denaturation produced by irradiation, it was compared with another type of denaturation that has been more extensively studied. In 1925, I<sup>10</sup> showed that the denaturation of serum albumin by heat was, when carried out under certain specified conditions, reversible. Wells and Lewis<sup>11</sup> previously had reached a similar conclusion from their experiments on the sensitization of guinea-pigs to heat-coagulated protein. Besides this, some authors, as Emden,<sup>12</sup> believe that the irreversibility of certain colloidal changes bears a direct relationship to biologic age and death. It is therefore of considerable interest to compare proteins denatured by irradiation and by heat and to determine whether the changes in the protein produced by irradiation are reversible or not. Experiments

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4. Spiegel-Adolf, M.: *Strahlentherapie* **89**:367, 1928.

5. Fernau, A., and Spiegel-Adolf, M.: *Biochem. Ztschr.* **204**:14, 1929.

6. Spiegel-Adolf, M., and Pollaczek, K. F.: *Biochem. Ztschr.* **214**:175, 1929.

7. Harris, D. T.: *Biochem. J.* **20**:271, 280 and 288, 1926.

8. Spiegel-Adolf, M.: *Biochem. Ztschr.* **197**:197, 1928.

9. Spiegel-Adolf, M., and Fernau, A.: Unpublished observations.

10. Spiegel-Adolf, M.: *Biochem. Ztschr.* **170**:126, 1926.

11. Wells, H. G., and Lewis, J. H.: *J. Biol. Chem.* **59**:3, 1924.

12. Emden: *Klin. Wchnschr.* **8**:1913, 1929.

carried out for this purpose showed that there was an important difference between denaturation caused by heat and that due to irradiation. The latter proved to be an irreversible reaction when examined by the methods employed in the study of the denaturation due to heat. The results obtained were the same regardless of whether ultraviolet rays or radium had been used for the precipitation of the protein. In either case the precipitates formed exhibited properties that were distinctly different from those of albumin precipitated by heat.

4. If, instead of electrolyte-free solutions of proteins, solutions are used that contain small amounts of acid or of alkali, different effects are obtained by irradiation. In all of these experiments, the amount of irradiation is sufficient to cause complete precipitation in an electrolyte-free solution of protein of the same concentration. Such solutions are completely denatured by irradiation even in the presence of electrolytes. In these cases, however, precipitation occurs only when the concentration of acid or alkali in the solution is too small to dissolve the denatured protein. (I was able to show that the acid-binding capacity of serum albumin was not affected by denaturation with the mercury arc. This means that the hydrogen ion concentrations of such solutions do not change under treatment with rays of short wavelength.) The same solutions exhibit a similar behavior when heated. Although irradiation may produce no visible change in solutions that contain sufficient acid or alkali, the fact that a change has taken place may be demonstrated in various ways. Removal of the acid or of the alkali by dialysis or electrodialysis results in a flocculation of both the heated and the irradiated protein. Although small differences in color and even in odor may be detected, it is difficult to demonstrate by chemical methods that the protein has suffered any change. That such changes have taken place may, however, be indicated by optical methods.

5. Solutions of protein exhibit absorption bands in the ultraviolet region of the spectrum. The addition of small amounts of acid or alkali, sufficient to prevent coagulation of the protein during irradiation, does not affect these absorption spectrums. When, however, such solutions are exposed to the mercury arc or to the rays of radium, a marked increase in the absorption of light of short wavelength may be observed<sup>13</sup> (figs. 1 and 2). These changes may be confirmed and demonstrated in a different way. For this purpose, Hausmann and Spiegel-Adolf<sup>14</sup> made use of a small ring of glass fixed to a quartz

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13. Spiegel-Adolf, M., and Krumpel, O.: *Biochem. Ztschr.* **28**:190, 1928.

14. Hausmann, W., and Spiegel-Adolf, M.: *Klin. Wchnschr.* **6**:2182, 1927.



plate and divided by a small piece of glass into two separate compartments. One of these compartments was filled with a solution of irradiated protein, while the other contained a nonirradiated control. The entire apparatus was then placed on the forearm of a man and exposed to the mercury arc for fifteen minutes. After this period, and for some hours afterward, the section of skin covered with the nonirradiated

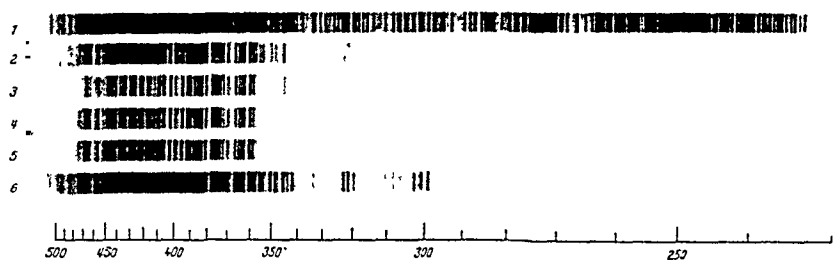


Fig. 1.—Influence of ultraviolet rays on the absorption spectrums of solutions of protein (the concentration of protein is the same in all solutions): (1) iron spark; (2) control acidified solution of serum albumin; (3) control alkaline solution of serum albumin; (4) irradiated acidified solution of serum albumin; (5) irradiated alkaline solution of serum albumin, and (6) nonirradiated, electrolyte-free solution of serum albumin.

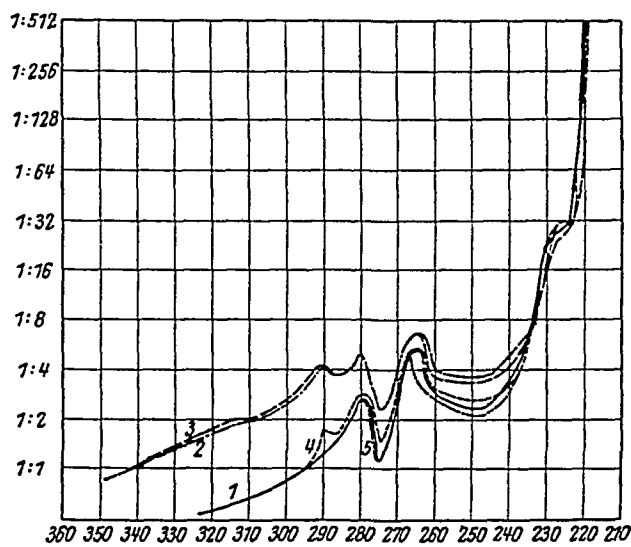


Fig. 2.—Absorption curves of solutions of protein: (1) pure serum albumin; (2) irradiated acidified solution of serum albumin; (3) irradiated alkaline solution of serum albumin; (4) control solution of alkaline serum albumin, and (5) control solution of acidified serum albumin. The ratios at the left represent the concentrations of protein; the numerals at the bottom, the wavelengths in millimeters.

protein showed a distinct erythema (fig. 3). That part of the skin covered by the irradiated protein showed either no change or only a very slight change, thus proving that irradiated proteins are less per-

meable to light of short wavelength than nonirradiated ones. These experiments have been repeated, with similar results, by various investigators. It has even been suggested that the increased absorption of light by irradiated proteins may have some bearing on those cases in which, after repeated exposure to the mercury arc, the skin becomes insensitive to further exposure without any pigmentation having occurred. The same experiment was repeated, with use of cultures of bacteria and agar containing red blood corpuscles instead of the skin, with similar results. Together with Oshima,<sup>15</sup> I was able to show by means of this method that the growth of various kinds of bacteria was arrested by rays of different wavelengths.

It was shown by Lewis<sup>16</sup> that the various serum proteins absorb different amounts of ultraviolet rays. The globulins exhibit the great-



Fig. 3.—Effect of irradiation on the permeability of a solution of protein to ultraviolet rays: *A*, portion of skin covered by quartz alone; *B*, portion of skin beneath control solution of protein, and *C*, portion of skin beneath irradiated solution of protein.

est absorption, while the albumins show the least. Pseudoglobulin exhibits an absorption intermediate between the other two. When these proteins are irradiated, however, these differences in absorption disappear almost completely, for the albumins exhibit the largest increase in absorption, while the globulins show the least.<sup>8</sup> This does not mean that irradiation transforms albumins into globulins, as occasionally indicated in the literature. It means merely that the albumins,

15. Spiegel-Adolf, M., and Oshima, Z.: *Biochem. Ztschr.* **208**:32, 1929.

16. Lewis, S. J.: *Proc. Roy. Soc., London* **93**:178, 1921-1922.

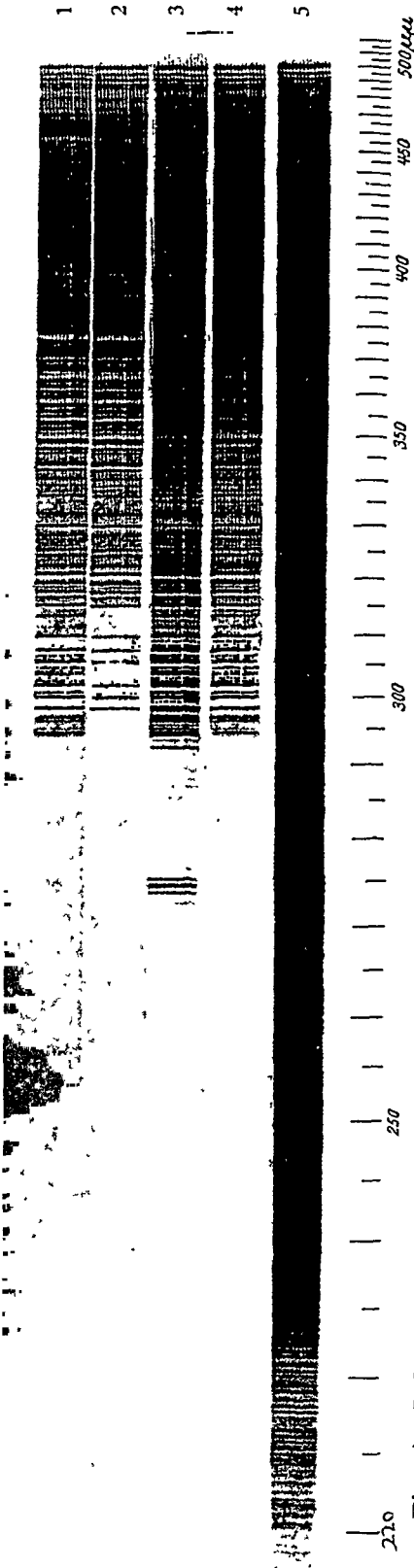


Fig. 4.—Influence of radium on absorption spectrums of solutions of protein: (1) control solution of alkaline serum albumin; (2) solution of alkaline serum albumin irradiated by radium; (3) solution of alkaline serum albumin diluted with water (dilution of 1:1); (4) solution of alkaline serum albumin of same concentration as solution 3 irradiated by radium, and (5) empty cell.

when irradiated, acquire some of the physicochemical properties of the globulins. They lose, either completely or in part, their solubility in water, and the colloidal character of their solutions changes from a hydrophilic to a hydrophobic one. They may be salted out of solution by smaller concentrations of salt in much the same way that the primarily hydrophobic globulins usually are. Proteins exposed to the rays of radium behave in exactly the same way; i. e., they become more opaque to light of short wavelength<sup>17</sup> (fig. 4). It may seem at first glance, that radium exerts a lesser effect on proteins than does the

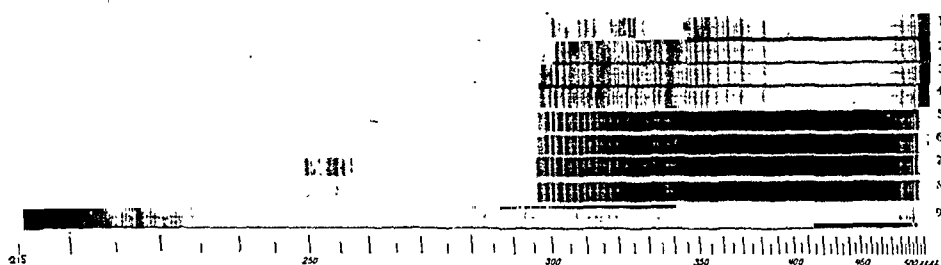


Fig. 5.—Influence of heat on absorption spectrums of concentrated solutions of proteins: (1) alkaline solution of serum albumin, boiled; (2) alkaline solution of serum albumin, unheated; (3) acid solution of serum albumin, boiled; (4) acid solution of serum albumin, unheated; (5) alkaline solution of egg albumin, boiled; (6) alkaline solution of egg albumin, unheated; (7) acid solution of egg albumin, boiled; (8) acid solution of egg albumin, unheated, and (9) empty cell.

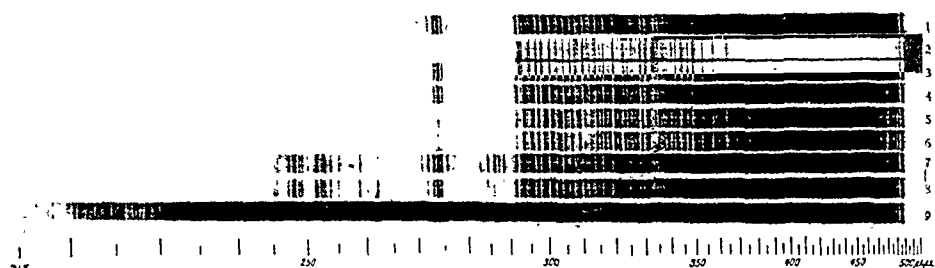


Fig. 6.—Influence of heat on absorption spectrums of dilute solutions of protein: (1) alkaline solution of serum albumin, unheated; (2) alkaline solution of serum albumin, boiled for fifteen minutes; (3) alkaline solution of serum albumin, boiled for one hour; (4) acid solution of serum albumin, unheated; (5) acid solution of serum albumin, boiled for fifteen minutes; (6) acid solution of serum albumin, boiled for one hour, and (9) empty cell.

mercury arc. The exposure used in the case of radium was, however, not long enough to permit its effects to be compared quantitatively with those produced by ultraviolet rays.

17. Spiegel-Adolf, M., and Krumpel, O.: *Biochem. Ztschr.* **190**:28, 1929.

It was indicated that the denaturation of electrolyte-free solutions of protein produced by heating differed from that caused by irradiation in that the former process was reversible. This criterion for differentiating between the two types of denaturation cannot be applied to proteins denatured in the presence of alkali, because, under these conditions, the heated protein also undergoes an irreversible change. Differences between alkaline solutions of protein denatured by heat and those denatured by irradiation may, however, be detected easily with the aid of the quartz spectrograph. It is observed (fig. 5) that with the same concentration of protein, there is no increase in absorption produced by heating the solution. With higher dilutions of protein, slight changes in absorption may be detected (fig. 6). These changes are not, however, at the same wavelength as before, and in some of the solutions there is no increase in absorption, but rather the reverse. Some of the solutions become even more permeable to light of short wavelength when heated.<sup>17</sup> Solutions of heated protein cannot, therefore, be used to filter ultraviolet rays, as in the experiments with irradiated protein. In all of the experiments mentioned, the solutions of protein were heated for fifteen minutes at the temperature of boiling water. Increasing the time of heating to one hour had no effect on the results obtained.

6. Since the results obtained by spectrographic examination are of considerable importance in the study of the constitution of chemical substances, it was considered worth while to seek other methods to check the results described. For this purpose I made use of another optical method. The optical rotation of the substance under examination is measured with light of several different wavelengths, and the results obtained are plotted on a diagram in which the abscissa represents the square of the wavelength, and the ordinate the reciprocal of the specific rotation. For simple substances, it is found that the plotted values lie on a straight line that intersects the abscissa at a point that represents the wavelength of the chief absorption band of that particular substance. This method, used by Lowry and Dickson, is based on the equations of Drude. It was applied to solutions of proteins subjected to ultraviolet rays, radium and heat.<sup>18</sup> It was found that the points of intersection of the various curves with the abscissa were not affected by the addition of sufficient alkali to prevent coagulation of the protein. This result is in accordance with the conclusions drawn from the spectrographic investigations. It was found, also, that heating the solution of protein had no effect on the inter-

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18. Spiegel-Adolf, M.: *Biochem. Ztschr.* **213**:475, 1929.

section of its curve with the abscissa (fig. 7). The quotient between the specific rotations at certain specified wavelengths, known as the dispersion quotient,<sup>19</sup> remains at the value characteristic for the unheated solutions. Irradiated protein, on the other hand, exhibits an entirely different behavior. Although the curve of optical dispersion is still a straight line, it intersects the abscissa at a point that indicates a longer wavelength for the characteristic absorption band. These results are in direct agreement with those obtained by spectrographic examination. The dispersion quotient reaches a value usually observed in ketones derived from amino-acids by oxidation. In the first experiments, the protein was denatured by ultraviolet rays. Further experiments on the effects of radium on the same protein led to practically the same results.

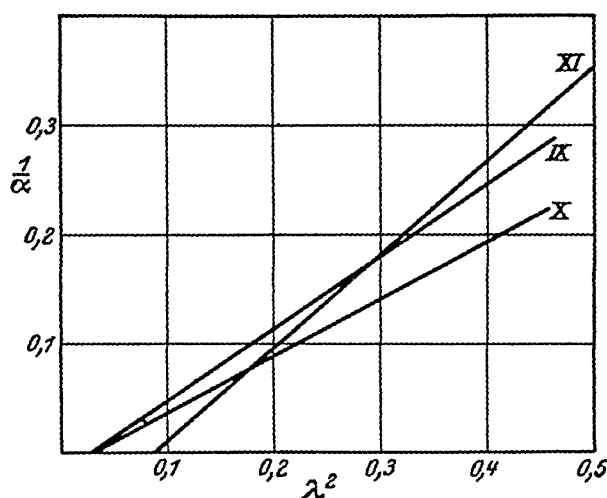


Fig. 7.—Optical dispersion of heated and irradiated alkaline solution of pseudoglobulin: IX, control alkaline solution of pseudoglobulin; X, alkaline solution of pseudoglobulin, heated, and XI, alkaline solution of pseudoglobulin, irradiated.

7. Experiments were also carried out on the immunologic properties of the heated and irradiated proteins.<sup>20</sup> In the presence of sufficient acid to prevent coagulation of the protein, it was possible to show that solutions that had been heated gave precipitates with serums against the unheated protein, whereas solutions irradiated with the mercury arc failed to give any reactions. When heated proteins were used for immunization, the antisera obtained also reacted with unheated protein. When irradiated proteins were used for immunization, however, three out of four sera obtained failed to react with the untreated protein.

19. The dispersion quotient is  $\frac{[\alpha]_F}{[\alpha]_C}$

20. Spiegel-Adolf, M., and Higuchi, S.: Unpublished observations.

8. The results of the investigations lead to two principal conclusions: First, as shown by means of the various independent methods, rays of short wavelengths produce effects on proteins that differ from those observed when the protein solutions are heated to 100 C. Second, various types of rays, such as ultraviolet rays, x-rays and radium, produce effects that are qualitatively, though not quantitatively, similar. These conclusions are applicable only to proteins. It was pointed out that coagulation of the protein results from a few minutes of irradiation with the mercury arc and that three hours' exposure to x-rays (72 Holzkecht units) and nine hours' exposure to 80 mg. of radium are required to produce the same results. This suggests that only a small part of the energy of the x-rays and of radium is active in the coagulation of the protein, whereas most of the ultraviolet rays are absorbed by the protein. This may be due to the fact that the principal absorption bands of the solutions of proteins lie in this region of the spectrum. If, therefore, it were possible to convert part of the energy of radium and of x-ray radiations into ultraviolet rays, a possibility which is in accordance with Stokes' law, the effects of these radiations on proteins should be intensified. For this purpose, I made use of the property of certain crystals to fluoresce in the ultraviolet region when irradiated by radium or x-rays. Salts of tungstic acid were not used because I wished to avoid the production of light of longer wavelength.<sup>21</sup> The crystals used were enclosed in quartz tubes to avoid contact with the liquid. By means of this method, I was able to show that whereas proteins irradiated by x-rays and radium without ultraviolet fluorescence exhibit no changes, the same proteins similarly treated in the presence of the fluorescent material are coagulated.<sup>22</sup> Similar experiments were also carried out with red blood corpuscles and with such living cells as *Paramecium*. The first changes in red blood corpuscles produced by irradiation with x-rays appear in one-fourth the time when a quartz tube containing the fluorescent crystals is immersed in the suspension. *Paramecia*, which cannot be killed by radium, or which may be destroyed only with great difficulty, promptly died when irradiated under these conditions. In the case of the red blood corpuscles, almost the same effects are produced when filtered x-rays are used, indicating that ultraviolet rays may be called forth in such places, as tissues, where it can not penetrate from the outside. Further investigations are necessary to determine whether this method is of any therapeutic value. I am at present studying its application to tumor tissues in mice. The well known results of ultraviolet rays in tuberculosis suggest an attempt in this direction.

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21. Perrine, J. O.: *Physiol. Rev.* **22**:48, 1923.

22. Spiegel-Adolf, M.: *Klin Wchnschr.* **9**:1615, 1930.

# PSEUDOHERMAPHRODITISMUS MASCULINUS EXTERNUS ASSOCIATED WITH SUPRARENAL HYPERTENSIA AND VASCULAR HYPERTENSION

REPORT OF A CASE \*

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Fairly well defined clinical syndromes are known to exist in association with malformations of the suprarenal gland, and since experimentally it is impossible to reproduce many of these, it becomes increasingly important to record those instances in which clinical findings may be correlated with postmortem observations in order that recognition of the syndromes may be expedited and insight into essential relationships deepened. The case submitted here is of exceptional clinical, as well as pathologic, interest. The patient, a person 26 years old, to all outward appearances a female, had not undergone the changes associated with puberty and had had for a known period of six years a high blood pressure, which led to her sudden death from spontaneous intracerebral hemorrhage. Postmortem examination disclosed a remarkable degree of hyperplasia of suprarenal tissue associated with pseudohermaphroditismus masculinus externus, marked cardiovascular changes and apparent absence of the pineal gland.

## REPORT OF CASE

*Clinical History.*<sup>1</sup>—The patient, a school teacher in the twenties, was a young person of unusual mental attainments, an excellent scholar, who was studying for the degree of Doctor of Philosophy in history. She was very nervous and excessively vivacious in her speech, but was not irritable. Although 6 feet tall (183.5 cm.), she was handsome, essentially feminine, not only in appearance, but also in her reactions to, and her relations with, her external environment. She was admitted to Barnes Hospital on April 23, 1923, during a fairly typical attack of appendicitis, several of which she had had before. No operation was performed. She gave a history of having had recurrent attacks of heart trouble with "dropsy" since her childhood. She stated that she menstruated once when she was 15 years old, but had not done so since. The menses, she stated, were scanty and lasted one day. Subsequently she never had any monthly pain or cramplike sensations. She occasionally had nosebleed in childhood. Physical examination

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\* From the Department of Pathology of the University of Chicago and the Illinois Central Hospital.

1. The Barnes Hospital in St. Louis gave us access to the clinical record.



revealed a tall, fairly well nourished white woman who was very restless and fidgety. The skin was rather dry, and there was a marked absence of axillary and pubic hair. The breasts were very small and infantile. A soft systolic murmur, best heard at the apex of the heart, was not transmitted to the axilla. The apical impulse was in the fourth interspace 9 cm. from the midsternal line. The eye-grounds were normal. The blood pressure varied from 180 systolic and 140 diastolic to 150 systolic and 98 diastolic. So much muscular resistance was encountered that accurate palpation of the pelvic organs was impossible. It was thought best not to disturb the patient further at the time, but to make pelvic examination under anesthesia should the need become urgent. A lateral stereoscopic x-ray picture of the skull suggested an oxycephalic syndrome and increased intracranial pressure. There were albumin and hyaline and granular casts in the urine. There was some renal insufficiency. The blood chemistry was normal. The patient was discharged on May 4, 1923. On June 10, 1923, she reentered Barnes Hospital prior to a tonsillectomy which had been advised on her previous admission. The blood pressure varied from 195 systolic and 145 diastolic to 175 systolic and 120 diastolic. On the second day following the operation an attack of gastric tetany developed. The blood pressure dropped to 100 systolic and 60 diastolic, and uremic symptoms appeared. The nonprotein nitrogen rose to 111 mg., and the urine contained albumin and many hyaline and granular casts. The renal function showed marked impairment. Subsequently the uremic symptoms cleared, and the blood pressure rose. At the time of the patient's discharge, on June 25, 1923, the blood pressure had risen to 155 systolic and 105 diastolic. During the following summer the patient had occasional severe headaches. The hands and eyelids were swollen at times, especially in the mornings and evenings. She tired easily. On Sept. 10, 1923, she reentered Barnes Hospital for a reexamination. The renal function was improved, and the level of nonprotein nitrogen was lower. Following a series of tests the blood pressure rose to 210 systolic and 180 diastolic, and the patient had a severe headache with nausea and vomiting. An electrocardiographic tracing showed no abnormalities. During the remainder of the patient's stay in the hospital the blood pressure stayed constantly around 190 systolic and 158 diastolic. The urine showed a trace of albumin and a few white blood cells and granular casts. The nervousness and excitability were apparently increased. The patient was discharged on Sept. 17, 1923. Later she refused treatment with ovarian extract for infantilism. Friends stated that she had severe headaches at frequent intervals. The clinical diagnosis at this time was chronic diffuse nephritis, hypertension, oxycephaly, hypogonadism and infantilism. In 1928, the patient was acting as dean and teacher of history in a girl's school and was under some strain about her work. Death came suddenly while she was in consultation with the head of her school. She slid down from her chair unconscious, but breathing, and died within a few minutes.

*Necropsy.*—Necropsy was performed at the Illinois Central Hospital in Chicago. The examination disclosed a well developed, well nourished white woman, looking about 26 years of age, weighing 140 pounds (63.42 Kg.) and having a height of 6 feet, 1 inch (185.42 cm.). There was a slight amount of hair on the face, a few hairs on the labia, but no pubic hair. The external genitalia resembled those of a preadolescent girl. The breasts were poorly developed. On the right side a testicle, 2.5 cm. long and 1.1 cm. wide, with a rudimentary epididymis, lay in a sheath in the abdomen retroceally (fig. 1). On the same side a vas deferens, 11 cm. long, apparently entered the wall of the bladder just posterior to the urethral attachment. At this point there was a small mass of solid tissue,

resembling muscle, measuring 2 by 2 by 0.6 cm., which was loosely attached to the wall of the bladder. No uterus or ovaries were present. The vagina was 3 cm. long and 1 cm. wide and ended blindly (fig. 2). There was a slight nodular thickening at the apex of the vagina which might correspond to the cervix uteri. A rudimentary round ligament was present.

The suprarenal glands were greatly increased in size and together weighed 46.5 Gm. The left suprarenal gland was in the normal position. It was 7 cm. long, 4 cm. wide, and from 0.5 to 1 cm. thick. The right was 8 cm. long, 4.5 cm. wide and 1 cm. thick. At the lower pole of the right suprarenal gland there was a nodule that was dark reddish brown flecked with yellow. It measured 2 cm. in length and 1 cm. in width.

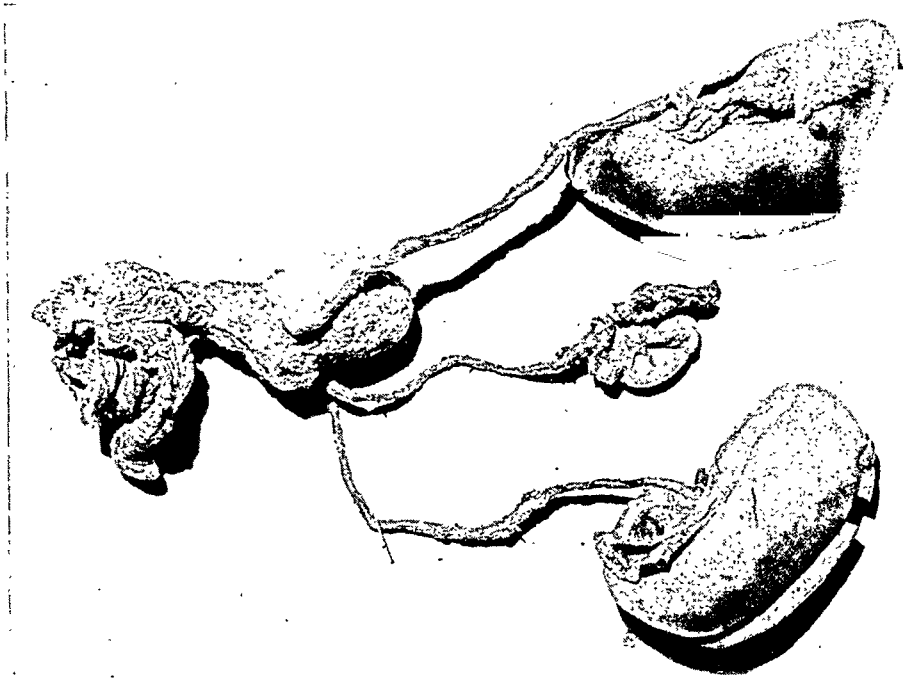


Fig. 1.—The preadolescent testicle and epididymis which lay behind the cecum. From it the vas deferens passes to end blindly in a mass of undifferentiated muscle tissue lying behind the bladder in the usual site of the prostate. Note the very small size of the bladder.

There was a marked hyperplasia of the intestinal lymphoid follicles and mesenteric lymph nodes. The spleen weighed 380 Gm., and the germinal follicles were unusually prominent.

The left kidney was about the normal size and was edematous. The cortex was slightly narrowed, and the markings were indistinct. The capsule stripped easily, leaving a smooth surface. The pelvis seemed normal. The right kidney was somewhat smaller, but similar to the left. The bladder was extremely small. It measured 4 cm. in its greatest external diameter, and the wall was 1 cm. thick, so that the cavity was extremely small. The urethral wall was greatly thickened.

The liver weighed 2,420 Gm. and was a hand's breadth below the costal margin. It was dark reddish brown. No other significant changes were noted in the abdominal cavity.

The heart weighed 430 Gm. The apex of the heart was made up entirely of the left ventricle. The left ventricular wall measured from 16 to 22 mm., and the right from 2 to 5 mm., in thickness. There were no valvular lesions, except for tiny warty vegetations on the corpora arantii of the aortic cusps and on the mitral leaflets, which were otherwise smooth and shining. There were small atheromatous patches in the coronary arteries, which were unobstructed. Small atheromatous patches were present on the arch of the aorta, and yellow and white atheromatous patches at the opening of the abdominal vessels. The aorta in its thoracic and abdominal portions was normal in size.



Fig. 2.—Bladder and vagina. The greatly thickened wall of the bladder with its small cavity is seen above (A). Behind and below is the vas deferens (B), ending blindly in a mass of muscle tissue behind the bladder at the site of the prostate. The rudimentary vagina lies open (C).

There were marked bilateral pulmonary hyperemia and edema. The thyroid gland weighed 27.5 Gm. and contained glistening colloid. The thymus gland was not mentioned. There were no other significant changes in the thorax. A generalized arteriosclerosis was present.

There was no evidence of external trauma to the skull. The brain was edematous; the convolutions were flattened and the sulci narrowed. There were hemorrhages in the leptomeninges over the pons and extending down into the spinal canal. An extensive hemorrhage led on the right to a total interruption of the connection of the cortex with the internal capsule. Of the basal ganglions on the right, only a small medial part containing the anterior part of the internal

capsule was present. The left basal ganglions showed flattening of the nucleus caudatus and an outward convex curve of the internal capsule. The basilar and vertebral arteries displayed an astounding degree of arteriosclerosis. The hypophysis appeared grossly normal. The pineal gland was not definitely recognizable.

The microscopic examination of the tissues revealed conditions as follows:

**Suprarenal Glands:** In sections stained with hematoxylin and eosin, the fibrous capsule of the suprarenal gland was not thickened. The zone glomerulosa was narrow and in places was entirely absent. In many regions the characteristic arrangement of this zone in spherical or oval groups of epithelial cells was lost, the epithelium being definitely compressed between the capsule and the hyperplastic cells of the deeper cortical layers. Where not compressed, the epithelial cells of the zona glomerulosa were columnar. Their cytoplasm stained lightly with eosin and was somewhat granular, and the nuclei were rich in chromatin. In an occasional region, cells from the zone glomerulosa appeared to invade the capsular tissue immediately adjacent to the gland. The characteristic arrangement of the zona fasciculata and the zona reticularis in long columns and irregular anastomosing cords was, for the most part, lost, although an occasional suggestion of fascicular arrangement was present in the zona fasciculata. The epithelium consisted of large, irregularly arranged, polyhedral cells with an acidophilic cytoplasm and spheroidal, vesicular nuclei which varied somewhat in size and were less rich in chromatin than were those in the zona glomerulosa. Many of these cells had a distinctly foamy cytoplasm, and many others toward the inner layers of the cortex were heavily pigmented with a brownish granular pigment. There was an enormous diffuse hyperplasia of the epithelium of these two layers, which could not be distinguished one from the other. In an occasional region, there was a tendency toward encapsulation of small nodular groups of cortical epithelium by fibrous tissue. The cortical cells were seen to invade the glomerular zone, in regions crowding it aside and becoming adjacent to the capsule, at times invading this structure, and occasionally forming small extracapsular adenomatous nodules the substance of which was continuous with that of the gland. In regions this extra-capsular tissue was not limited by a capsule of its own, but infiltrated the adipose tissue (fig. 3). The medulla was likewise invaded by cortical epithelium, and in places islands of cortical tissue were present in the medulla. There was no apparent increase in connective tissue stroma or in vascularity. Regions of the suprarenal gland were distinctly vacuolated, being composed of signet ring cells resembling those of adipose tissue. There were occasional foci of lymphoid cells, the largest one of which contained many cells with large eosinophilic granules and an occasional mononuclear giant cell. Outside the capsule there were many circumscribed nodules of cortical tissue. In places these cells were seen to invade the adipose tissue, not being limited by a capsule.

There was an extensive diffuse increase in medullary tissue, the cells of which were polyhedral or irregularly oval, and were arranged with marked irregularity. The nuclei were spherical or slightly ovoid, were somewhat vesicular, and contained a moderate amount of chromatin. The cytoplasm was somewhat basophilic and slightly granular. A few of the cells possessed foamy cytoplasm and others contained brownish granular pigment. In regions there was a slight infiltration by the signet ring cells. The medullary tissue had a bizarre arrangement. In places the medulla was very narrow; in others it became irregularly widened. Some regions appeared as islands and streaks of cells completely surrounded by cortical tissue. In others the cortical cells were diffusely infiltrating the medullary tissue. The medullary stroma seemed normal in amount, and there was no increase in vascularity. In sections stained with van Gieson's connective tissue

stain, there appeared an increase in connective tissue in the occasional regions where there was an attempt at nodular arrangement and encapsulation of the cortical epithelium. In one region in the medulla, the stroma formed relatively heavy connective tissue strands. Aside from that in the region mentioned the connective tissue was not increased.

**Testis:** In sections stained with hematoxylin and eosin, the testis was surrounded by a delicate fibrous tunica albuginea, which projected into the gland at one point, forming the mediastinum or hilus testis. The tubules appeared to have a definite fibrous tissue basement membrane, were small, and except for an occasional suggestion of a lumen were closely packed with epithelial cells resembling

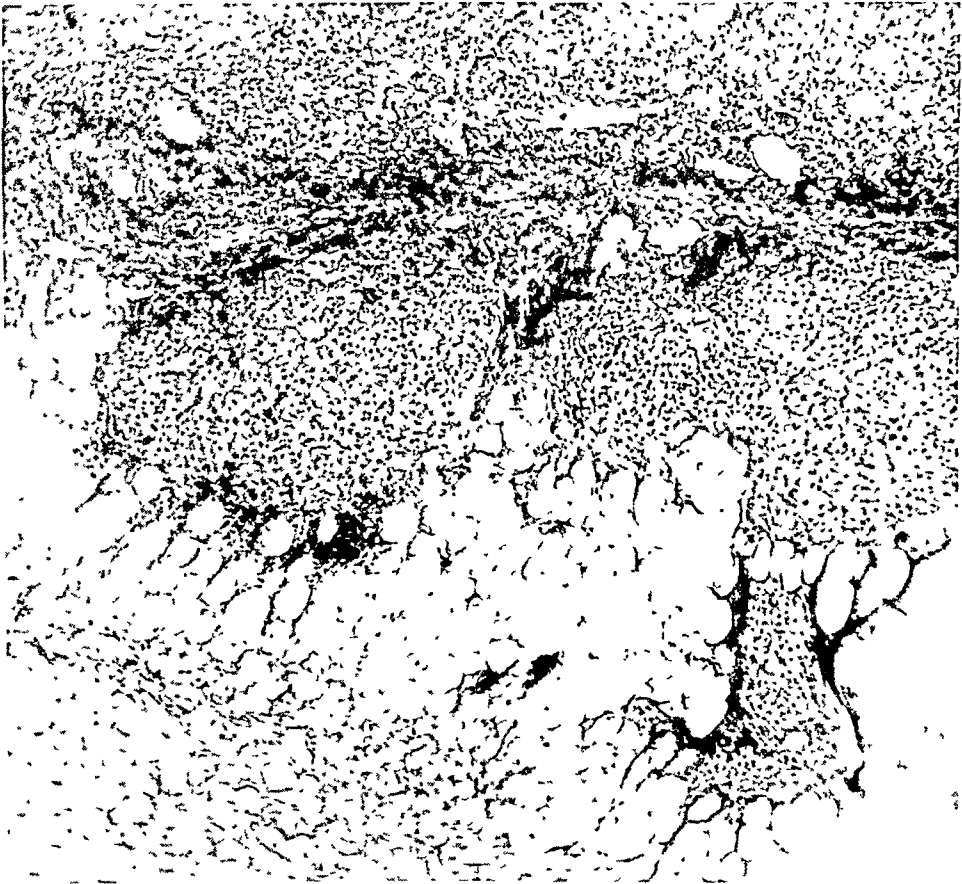


Fig. 3.—Suprarenal gland showing invasion of the adjacent adipose tissue by outgrowth of cells of the suprarenal cortex, infiltrating like neoplastic tissue, although the cells seem to be normal cortical cells;  $\times 75$ .

Sertoli cells of an embryonic testicle (fig. 4). Toward the capsule the tubules were most numerous, but were widely separated and irregularly scattered throughout the substance of the gland, which was made up almost entirely of large polygonal cells, the cell outlines of which were often indistinct (fig. 5). The nuclei were round or slightly oval and were pale and vesicular. The cytoplasm was more acidophilic than that of the tubular epithelium and was often finely vacuolated. The cells exhibited no pigmentation and resembled Leydig cells. In one region in a subcapsular position there was a small, circumscribed nodule apparently composed of closely packed tubules filled with a mass of cells having round or oval, hyperchromatic nuclei (fig. 4). The cell outlines could not be

distinguished. The van Gieson stain showed a fairly heavy connective tissue stroma in the nodule. Throughout the substance of the gland the van Gieson stain demonstrated a stroma that was not appreciably increased in amount. Near the hilus of the gland there was a region where large vacuoles resembling fat cells were present in the substance of the gland. The epididymis was small and of infantile type, and occasionally a duct showed papillary epithelial proliferation. Mallory's stain showed only a delicate collagenous reticulum around the blood vessels and tubules. The epididymis was widely separated from the capsule of the testis by loose areolar connective tissue.

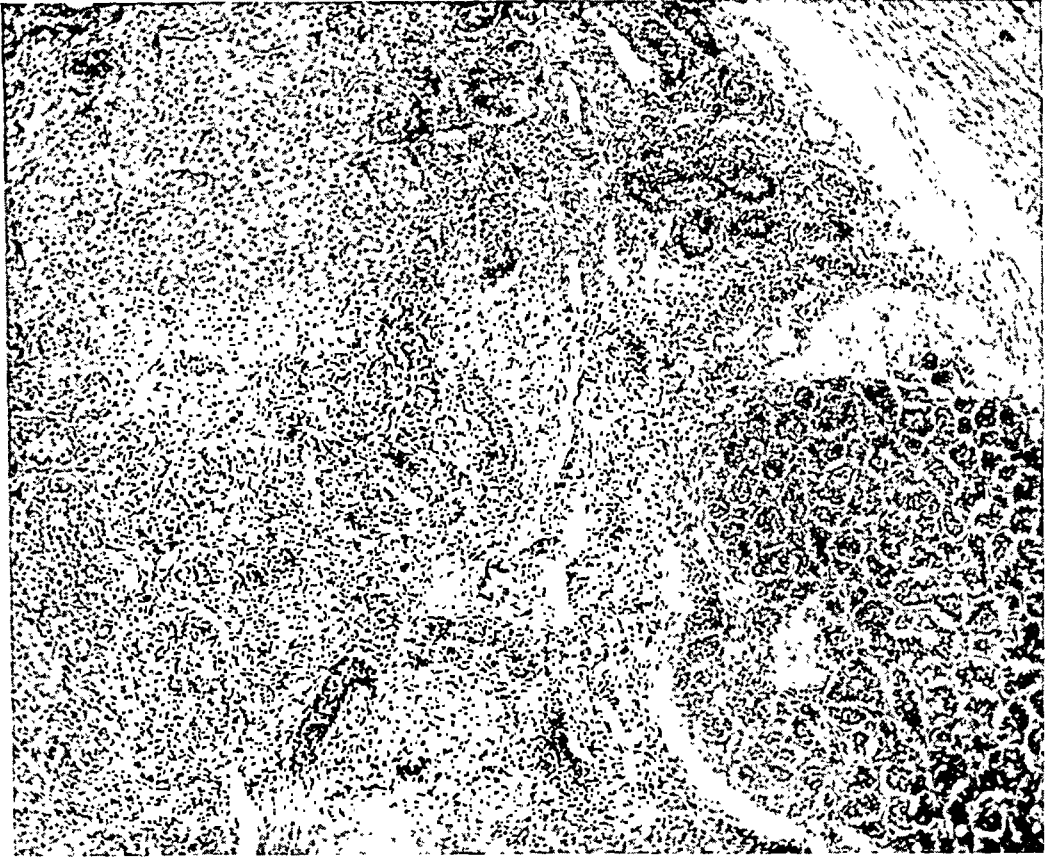


Fig. 4.—Testicle. The photomicrograph shows the general structure, with but few, undeveloped seminiferous tubules, a diffuse cellular intertubular growth and a nodule of undeveloped tubular structures;  $\times 75$ .

**Other Organs:** The nodule at the base of the bladder consisted of a mass of spindle cells in poorly defined bundles resembling underdeveloped nonstriated muscle fibers. No glandular elements were seen, and there was nothing to indicate whether this was a rudimentary prostate or uterus. The vagina showed no histologic changes.

The anterior lobe of the hypophysis showed a slight increase in connective tissue. The epithelial cords were often irregular in arrangement, sometimes apparently hypertrophied and containing more colloid than normal.

Serial sections through a small mass of tissue occupying the site of the pineal gland failed to reveal any trace of pineal tissue.

In the region of the cerebral hemorrhage, only the usual infiltration by blood was seen, there being no other changes recognizable in a preparation stained with hematoxylin and eosin, except an occasional blood vessel surrounded by cells filled with brown pigment as if from a previous small hemorrhage.

The thyroid gland showed no definite abnormalities.

Peyer's patches of the ileum exhibited tremendous hyperplasia, each follicle being greatly enlarged with a conspicuous germinal center. There was also a

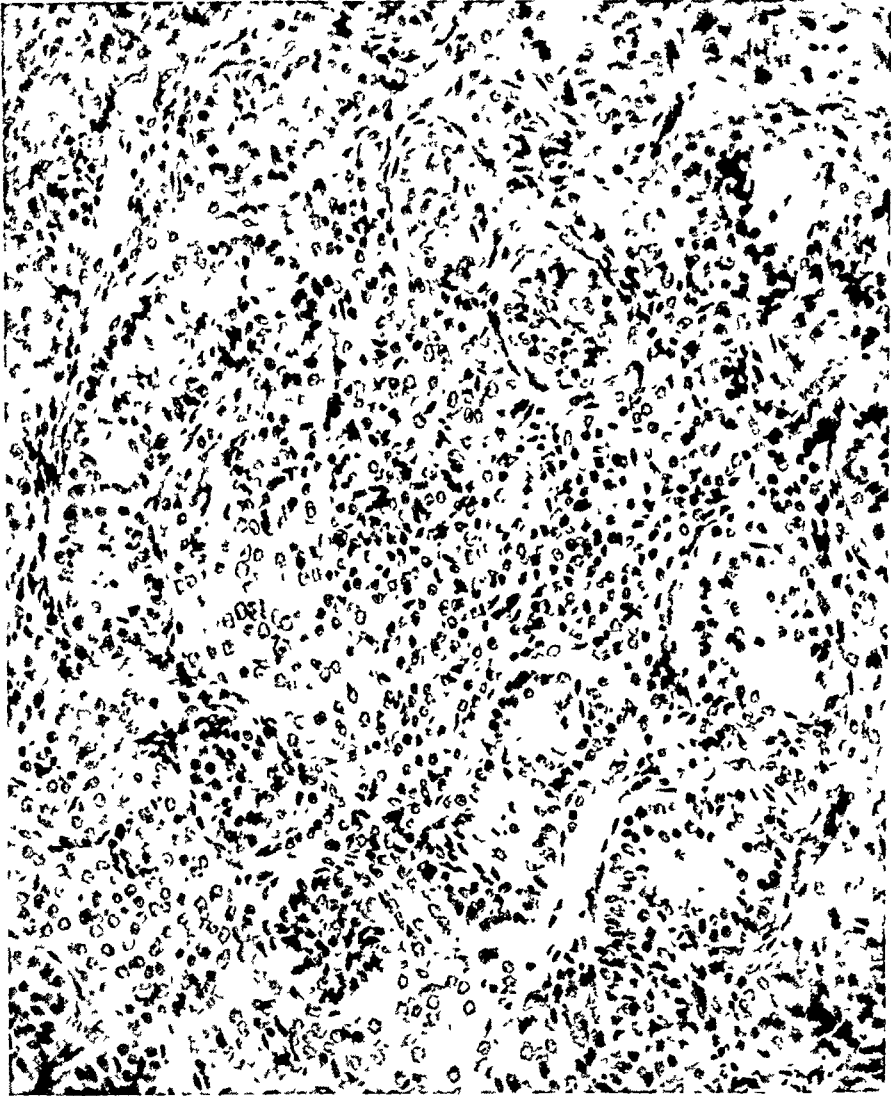


Fig. 5.—Testicle;  $\times 200$ . The photomicrograph shows the undeveloped state of the organ, which much resembles the testicle of an embryo in an early stage. The tubules are lined with cells resembling Sertoli cells. Between the tubules are sheets of cells resembling Leydig's cells as seen in undeveloped testicles.

diffuse lymphoid infiltration between the follicles. Otherwise the intestine was normal. The mesenteric lymph nodes showed marked lymphoid hyperplasia, giving rise to a picture that was distinctly "mulberry" in type. The spleen showed marked increase in the size of the malpighian bodies which had fetal characteristics.

The myocardium was somewhat hypertrophied, but otherwise showed no changes. The aorta showed thickening of the intima with but slight atheroma. The media was not changed. The arteries everywhere showed thickening of the intima without degenerative changes.

The kidneys showed occasional small areas of localized atrophy and fibrosis beneath the capsule, which did not usually extend deeply into the cortex. Between these areas the renal tissue was approximately normal. No evidence of old or recent inflammatory changes was found in the glomeruli outside of these arterio-



Fig. 6.—Ileum showing the great hyperplasia of the lymphoid tissue;  $\times 33$ .

sclerotic scars. A few collecting tubules contained calcified masses, apparently calcified epithelium. The arterial walls were greatly thickened, the small arterioles being often almost occluded and their walls hyalinized. These observations indicate that such renal changes as were present were the result of arteriosclerosis. There was no evidence of a primary glomerulonephritis.

The urinary bladder was extremely small and showed an enormous hypertrophy of the muscular coats, but otherwise appeared normal. The urethra was normal.

The liver and the pancreas showed no significant changes.



## COMMENT

*Classification of Pseudohermaphroditism According to Klebs.*—Neugebauer,<sup>2</sup> in his extensive monograph on pseudohermaphroditism, uses the generally accepted classification of Klebs, recognizing pseudohermaphroditism of two types, the masculine in which the gonads are testicles, and the feminine in which they are ovaries. Both groups are further subdivided into the internal, external and complete types. External pseudohermaphroditism occurs when only the external genitalia, internal when only the internal organs, and complete when both internal and external organs, are not properly developed. The case reported here is obviously one of pseudohermaphroditismus masculinus externus; that is, the gonad was the testicle and the external genitalia were feminine. This group is rarely found associated with hyperplasias and neoplasms of the suprarenal cortex, by far the larger percentage of such associations belonging to the feminine pseudohermaphroditic and suprarenal virilism group. It is significant that statistically approximately 15 per cent of all pseudohermaphrodites are estimated to have bilateral hyperplasia of the suprarenal cortex.

*Classification of Suprarenal Pseudohermaphroditism According to Gallais.*—Largely through the correlation of clinical and postmortem observations it has become recognized that malformations of the suprarenal cortex have some definite relationship to anomalies of sexual development. Credit is given to Bullock and Sequeira<sup>3</sup> for emphasizing this relationship, and although references to the probable relationship are to be found in the older literature, it is since their paper that a considerable literature has grown up about the subject. The clinical picture brought about in the presence of hyperplasia or tumor of the suprarenal cortex when related to sexual anomalies has been given by Gallais<sup>4</sup> the name "le syndrome génito-surrénal," and he has divided cases in which this syndrome appears into four groups based on the age of the person at the time of the appearance of the symptoms.

Group I, "le pseudohermaphroditisme surrénal," contains cases of true suprarenal pseudohermaphroditism, which are far more frequently of the feminine type, that is, the gonad is the ovary. Masculine pseudohermaphroditism also occurs in this group, but is rare.

Group II, "le virilisme surrénal," embraces cases occurring after birth in the prepuberty period, leading to virilism. This condition is

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2. Neugebauer, F. L.: *Hemaphroditismus beim Menschen*, Leipzig, W. Klinkhardt, 1908.

3. Bullock, W., and Sequeira, J. H.: *On the Relation of the Suprarenal Capsules to the Sexual Organs*, Tr. Path. Soc. London **56**:189, 1905.

4. Gallais, Alfred: *Le syndrome génito-surrénal; étude anatomo-clinique*, Thèse, Paris, 1912.

the most common and is found most frequently in girls. It gives rise in varying degrees to symptoms of abnormal development of the body with pubertas praecox (sexual precocity), obesity, hypertrichosis and hypertrophy of the clitoris. When this condition occurs in boys, pubertas praecox and obesity are present together with an accentuated virilism. The sexual precocity may be distinguished from ovarian and pineal precocity by the heterosexual hair, and the adiposity from that of pituitary lesions (Froehlich's syndrome) by the sexual precocity (Oppenheimer and Fishberg<sup>5</sup>).

Group III, "la forme menstruelle," includes cases occurring in women in adult life during the years of menstrual activity. Women with this condition show amenorrhea, obesity, hypertrichosis of the masculine type, enlargement of the clitoris and a frequent change of attitude toward the male type. That this group is small is brought out in a recent paper of Crosbie and Smith<sup>6</sup> concerning a woman who showed typical changes in secondary sex characteristics in relation to an adenoma of the suprarenal cortex. This case they believed to be only the third or fourth authentic instance on record in the Gallais third group, i. e., the adult in the child-bearing period. It is of great interest to note that removal of the tumor effected a reversion to the normal female habitus. More recently Bauer<sup>7</sup> reported the case of a woman 35 years of age apparently belonging to this group. Over a period of one and one-half years she gradually became more obese, began to show hypertrichosis, masculine in type, and had an associated amenorrhea. The sexual instinct disappeared. Gynecologic findings were normal. The blood pressure ranged from 175 to 185 systolic and was 110 diastolic. The woman was operated on for a suprarenal tumor, but none was found. After a short period of time she died, and autopsy revealed normal suprarenal glands with a narrow lipoid zone. The hypophysis was normal, and the kidneys showed slight arteriosclerosis (Bauer<sup>7</sup>). Adult men seldom show the sexual and somatic changes seen in children and in women. According to Rolleston<sup>8</sup> there is only one authentic recorded case of feminism occurring in an adult man with a tumor of the suprarenal cortex.

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5. Oppenheimer, B. S., and Fishberg, A.: The Association of Hypertension with Suprarenal Tumors, *Arch. Int. Med.* **34**:631, 1924.

6. Crosbie, A. H., and Smith, L. W.: Primary Tumors of the Suprarenal Capsule with Especial Reference to Adrenal Virilism, *J. Urol.* **19**:241, 1928.

7. Bauer, Julius: Ueber function des gesamten Nebennieren system ohne anatomischen Befund, *Wien. klin. Wchnschr.* **43**:582, 1930.

8. Rolleston, Humphrey: The Manifestations of Primary Tumors of the Adrenals, *West London M. J.* **31**:105, 1925.

Group IV, "la forme obstetricale," includes cases occurring during the period near and after the menopause. Here the clinical picture is indistinct. Adiposity is commonly present, and hypertrichosis may or may not manifest itself.

As emphasized by von Gierke,<sup>9</sup> changes in the suprarenal glands are not uniform in the single groups, nor can specific changes be made out in connection with certain symptoms. Likewise, all changes that have commonly been observed in connection with disturbed sexual development, hyperplasias, adenomas and hypernephromas, may also occur without malformations of the genital organs and clinical genital symptoms. Since, in certain instances, symptoms have disappeared with removal of the tumor, there is little room to doubt that morphologic changes in the suprarenal glands do at times and in some as yet unknown manner cause changes in the genital system.

*Influence of the Bisexual Character of the Ovary on Development of Pseudohermaphroditism.*—While the determination of pseudohermaphroditism is a very difficult problem, it is interesting to speculate on the possible influence of the bisexual character of the ovary on the development of such states. In origin and early development the ovary and the testis are identical. The medulla of the ovary develops from the primary sex cords arising from the germinal epithelium, and furnishes a complete homologue of the seminiferous tubules in the male. The ovarian cortex develops later from the cords of Pflüger, for which the male gonad never forms normally any homologue. The bisexual potentialities of the ovary are well illustrated in the female bird, which is peculiar in that the left gonad alone matures to form the ovary, while the right gonad persists in a rudimentary state and to some extent in an embryonic condition. This situation was made the basis of significant investigations by Domm.<sup>10</sup> If the left ovary is removed either in young or sexually mature fowls, the right rudimentary gland, in by far the largest number of instances, forms a testis-like body. At times an ovary or an ovotestis develops, such cases being regarded as evidence of accompanying formation of the ovarian cortex of the right gonad before the influence inhibitory to the right cortical development began to operate. On the left, if a small portion of medullary tissue remains at the time of removal of the gland, a testis-like body may develop from that residue; if a portion of cortical substance also remains, an ovary or ovotestis may be formed. There is a distinct masculinizing effect on the accessory organs of reproduc-

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9. von Gierke, E.: Ueber Interrenalismus und interrenale Intoxikation, Verhandl. d. deutsch. path. Gesellsch. **33**:449, 1928.

10. Domm, L. V.: New Experiments on Ovariectomy and the Problem of Sex Inversion in the Fowl, J. Exper. Zool. **48**:31, 1927.

tion and other sexual characteristics in the presence of the testis-like gonad that forms from the rudimentary right gonad after removal of the left ovary. In an analysis of the biologic factors involved in this situation, Lillie<sup>11</sup> stated, "with respect to endocrine organization the female thus appears essentially as an hermaphrodite in which the masculine component is never extinguished but merely quiescent." To quote Lillie further, "the evidence for the bisexual character of the female is as conclusive for mammals as for birds, and, while the experimental evidence is not complete, points to the same conclusion that it is the female in mammals as well as in birds that possesses bisexual characteristics." The sterile free martin in cattle studied extensively by Lillie<sup>12</sup> is the female of two-sexed twins. It is zygotically a female, modified by receiving through anastomosis of blood vessels of the two placentae the sex hormone of the male twin, which, circulating in both individuals, results in producing an organism with male gonads and internal genitalia approaching the male type, but with female external genitalia. The cortex of the ovary does not develop in such a female, being suppressed by the male sex hormone. Lillie stated that here "a gonad with a primary female determination may form a structure which is morphologically a testis through suppression of the cortex and overdevelopment of the medullary cords under the influence of the male sex hormone." The resulting situation indicates that "sex in mammals cannot be diagnosed by character of gonads alone. The unexpected result is reached that the external genitalia and the mammary gland are more reliable criteria of female sex than the internal parts."

In a discussion of the present case, Dr. C. R. Moore suggested that the anatomic structure presented by the free martin is curiously like that of the masculine pseudohermaphrodite reported in this paper. The influence that may have brought about a suppression of the ovarian cortex in early fetal life is entirely speculative. It may be that the development of the suprarenal hyperplasia and the suppression of the ovarian cortex were conditioned by the same force. Mathias<sup>13</sup> believed that the primary rôle in the genital maldevelopment belongs to the suprarenal cortex and calls the condition "interrenalismus," while von Gierke<sup>9</sup> uses the term "interrenal intoxication."

In the person under discussion, the gonad during embryonic life apparently secreted sufficient hormone to influence the development of

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11. Lillie, F. R.: The Present Status of the Problem of "Sex Inversion" in the Hen, *J. Exper. Zool.* **48**:175, 1926.

12. Lillie, F. R.: The Free Martin: A Study of the Action of Sex Hormone in the Fetal Life of Cattle, *J. Exper. Zool.* **23**:371, 1917.

13. Mathias, E., and Petzal, E.: Eine Weitere Beobachtung von Interrenalismus, *Klin. Wchnschr.* **5**:2313, 1926.

the vas deferens and a rudimentary epididymis, but except for the patient's extreme height, it is difficult to determine any evidence of male sex hormone secretion in extrauterine life. The patient seemed sexually neutral, physiologically as well as in her behavior. This is of particular interest in view of the large number of cells resembling Leydig cells observed in the testicle. As brought out by Moore,<sup>14</sup> the rôle of the Leydig cells in the production of the male sex hormone is by no means settled. It seems probable that they are not of the extreme importance commonly attributed to them.

Krabbe<sup>15</sup> did not believe that the cases of suprarenal tumor or suprarenal hyperplasia associated with virilism or pseudohermaphroditism prove any especial connection between the suprarenal glands and sexual development, and that this association may be just as well or better explained by the development of early embryonic life. He postulated that the male portion of the ovary, being intimately associated with the development of the suprarenal gland in the beginning stages, is absorbed in some instances by the gland and developed into a part of its structure. Later development and secretion of these misplaced cells cause male characteristics to develop in the female, bringing about a state of virilism or feminine pseudohermaphroditism.

Among the authors other than those cited whose papers on virilism and pseudohermaphroditism are valuable may be noted Glynn,<sup>16</sup> Brutschy,<sup>17</sup> Collett<sup>18</sup> and Scabell.<sup>19</sup>

*Previously Recorded Cases of Pseudohermaphroditismus Masculinus Externus Associated with Hyperplasia of the Suprarenal Cortex.*—On reviewing the literature, the recorded case appears to be the fourth of pseudohermaphroditismus masculinus externus associated with hyperplasia of the suprarenal cortex and the second such case occurring in an adult.

Brutschy<sup>17</sup> in 1920 reported the occurrence of a bilateral hyperplasia of the suprarenal glands and an accessory suprarenal gland in a 14 day old infant dying of an acute gastro-intestinal disturbance. The external genitalia were feminine in type with two labia, a small

14. Moore, C. R.: The Biology of the Mammalian Testis and Scrotum, Quart. Rev. Biol. **1**:4, 1926.

15. Krabbe, K. H.: The Relation Between the Adrenal Cortex and Sexual Development, New York M. J. **114**:4, 1924.

16. Glynn, E. E.: The Adrenal Cortex, Its Rests and Tumors, Its Relation to Other Ductless Glands, and Especially to Sex, Quart. J. Med. **5**:157, 1911.

17. Brutschy, Paul: Frankfurt. Ztschr. f. Path. **24**:240, 1920.

18. Collett, Arthur: Genito-Suprarenal Syndrome (Suprarenal Virilism) in a Girl One and a Half Years Old, with Successful Operation, Am. J. Dis. Child. **27**:204, 1924.

19. Scabell, Albert: Deutsche Ztschr. f. Chir. **185**:1, 1924.

clitoris and a flat vaginal pit, but no vagina. The internal genitalia consisted of two testicles which lay on either side in the proximal part of the inguinal canal. On the right testicle lay a small mass of accessory suprarenal tissue. Brutschy described a hard muscle-like mass that lay behind the neck of the bladder and into which led two long canals. There was no evidence of uterus or of tubes. The only other abnormal findings were a split uvula and an accessory spleen.

Krabbe<sup>20</sup> in 1924 described, in a new-born child who lived only fifteen hours, external genitalia that were essentially feminine. Internally on the right a small testis had descended into the labium. No testis was present on the left, but in the abdomen, apparently occupying the place of a second testis, there was a tumor of suprarenal cortex tissue which showed no division into the characteristic zones. No rudimentary uterus was present. In the testis and epididymis there were inclusions of cells that resembled those of the suprarenal cortex. Krabbe called attention to the fact that the interstitial cells of the testis may resemble closely the cells of the suprarenal cortex. The suprarenal glands themselves were small and of essentially normal structure. The infant presented in addition spina bifida, paralysis of both lower extremities and clubfeet.

Apparently the first reported case of pseudohermaphroditismus masculinus externus with hyperplasia of the suprarenal cortex in an adult was described by von Gierke<sup>7</sup> in 1928. A 62 year old woman had never menstruated and, although married, was childless. There had never been the least doubt concerning her sex. She underwent an operation for double inguinal hernia. In both hernial sacs were found bodies that macroscopically and microscopically resembled testicles. The ductus deferens was typical. Fourteen days following operation the patient died of pulmonary embolism. Necropsy disclosed a body with feminine distribution of hair, palpable firm breasts and external genitalia of entirely feminine appearance with large and small labia and a small clitoris. The vagina was 3 or 4 cm. long. The spermatic duct proceeded from the inguinal canal to its normal position behind the bladder, where there was also a microscopic seminal vesicle containing only desquamated cells and no spermatozoa. The ducts opened into the upper end of the vagina. There was present a structure that may have been a rudimentary prostate. The breasts showed microscopically the picture of a virgin mammary gland. There were adenomas of both suprarenal cortices. Neither a clinical record of the blood pressure nor an anatomic description of the condition of the heart or of the blood vessels was included in the report.

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20. Krabbe, K. H.: *Ferholdet Mellein Binyrebarktumores og Pseudohermaphroditisme*, Hospitalistid. **67**:651, 1924.

THE RELATION OF THE SUPRARENAL HYPERTROPHY TO THE  
HYPERTENSION

Hypertension has been observed as a concomitant of hyperplasias, adenomas and hypernephromas of the suprarenal cortex, as well as of hyperplasias and chromaffin cell tumors of the medulla. Oppenheimer and Fishberg<sup>5</sup> believed that, in certain instances, at least, there is a causal relationship between the hyperplasia or tumor and the hypertension. They cited as evidence thirteen cases recorded in the literature and added two of their own, one with necropsy and one in which the diagnosis seemed very probable, in which no anatomic cause other than a demonstrable suprarenal tumor could be brought forward in explanation of the hypertensive condition. Particularly interesting are two cases of Volhard's quoted by Fishberg in which the clinical picture was that of diffuse nephritis with albuminuria. In both instances after the removal of a hypernephroma, the hypertension disappeared. Convincing also are coincident findings of hypertension and suprarenal tumor in children with no other demonstrable lesion to which the hypertension might be attributed. These authors also bring forward considerable anatomic evidence from series of cases reported in the literature that "diffuse hyperplasia and circumscribed adenoma formation in the suprarenal cortex are exceedingly common in persons suffering from hypertension whether it is nephritic or essential in type." More recently Fishberg<sup>21</sup> brought the literature on the subject to date, adding a case reported by Strauss<sup>22</sup> in which a hypernephroma in an adult woman was accompanied by both virilism and hypertension for which there was no other demonstrable cause. He also cited four cases in which hypernephroma in children has been accompanied by both pubertas praecox and hypertension. Other series of cases are noted in which hyperplasia of the suprarenal medulla has been observed in connection with hypertension. In a critical review of the literature, the author traced the development and lack of support of the conception that the hypertension is due to hyperepinephrinemia. The theory that the cortex plays some rôle in the production of epinephrine is not proved, and the presence of epinephrine in the blood of hypertensive patients has never been satisfactorily demonstrated, although the epinephrine content of the suprarenal glands may be increased in conditions with high blood pressure such as hypertension and nephritis

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21. Fishberg, A. M.: *Hypertension and Nephritis*, Philadelphia, Lea & Febiger, 1930.

22. Strauss, H.: *Ueber Hirsutismus und Virilismus suprarenalis*, Deutsche med. Wchnschr. **52**:2112, 1926.

(Wells<sup>23</sup>). Rabin<sup>24</sup> recently reported a chromaffin cell tumor (pheochromocytoma or paraganglioma) of the suprarenal medulla, collecting reports of thirty such tumors from the literature and summarizing all of the available information concerning the related clinical histories and observations at necropsy. It is significant to note that in nine of the thirty cases and in his own case there was present hypertension independent of renal disease. In two additional cases no clinical reports were available, but there was pathologic evidence of hypertension. Paroxysmal hypertension was present in two other instances. In presenting a case of paraganglioma with diffuse arteriosclerosis and arterio-sclerosis, Biebl and Wichels<sup>25</sup> attributed the condition to a prolonged overproduction of epinephrine, concluding that epinephrine sclerosis in man differs for some reason from experimentally produced epinephrine sclerosis in animals. It seems more probable that the sclerotic changes observed by these authors were present as a result of the prolonged hypertension.

As in the production of the "syndrome genito-surréal," it is not known why by far the larger percentage of suprarenal tumors fail to give rise to hypertension. Fishberg<sup>20</sup> stated that "it seems possible that a disturbance in the relations between the suprarenals and other organs of internal secretion results in some unknown way in the vasoconstriction which produces the hypertension." At present knowledge concerning the relation of the suprarenal glands both to sexual disturbances and to hypertension is speculative.

#### THE PINEAL GLAND

Serial sections through the region of the pineal gland of the patient failed to disclose the presence of pineal tissue. In a discussion of changes in the pineal gland, Horrax and Bailey<sup>26</sup> pointed out that the chief theory concerning the function of the pineal gland is that a secretion from this organ inhibits puberty, and that at the time of puberty involutional changes in the gland inhibit the secretion, thus allowing sexual characteristics to develop. Destructive lesions of the pineal gland are known at times to be associated with precocious puberty in boys. No such proved case exists in association with precocious

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23. Wells, H. Gideon: *Chemical Pathology*, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

24. Rabin, C. R.: *Chromaffin Cell Tumor of the Suprarenal Maculla (Pheochromocytoma)*, *Arch. Path.* **7**:228, 1929.

25. Biebl, Max, and Wichels, Paul: *Physiologische und pathologische anatomische Betrachtungen im Anschluss an einen Fall von Paragangliom beider Nebennieren*, *Virchows Arch. f. path. Anat.* **257**:182, 1925.

26. Horrax, Gilbert, and Bailey, Percival: *Pineal Pathology*, *Arch. Neurol. & Psychiat.* **19**:394, 1928.



puberty in girls. On the other hand, as these authors showed, such tumors may be present with failure of development of precocious puberty. Zandren<sup>27</sup> in 1921 reported an instance of absence of the pineal body in a boy 16 years old. The child had developed normally to the age of 10, but none of the phenomena of puberty followed. The thyroid gland and the hypophysis seemed normal, but the structure of the testicle corresponded to that of a child of 2 years. Zandren postulated from this case that the main function of the pineal body is to initiate rather than to inhibit puberty. If the tumor of the pineal body is a pinealoma or an adenoma, it may be argued that an increased secretion initiates a precocious puberty, thus lending support to Zandren's theory. To quote Horrax and Bailey,<sup>28</sup> "unfortunately for this theory not all the tumors associated with the pubertas praecox are pinealomas, many are teratomas, and one might point again to similar tumors unaccompanied by precocious puberty." Further complicating the picture, the same authors reported a case in which a ganglioneuroma developing from the anlage of the pineal gland completely replaced the structure, no cells resembling the normal structure of the pineal body being present. The patient, however, a man 40 years old, attained a normal physical, mental and sexual development. Krabbe<sup>28</sup> described the occurrence in a female infant, 1 year old, of a pineal gland that had been transformed into a pouch of neuroglia with thin walls and without any sign of pineal cells. The infant did not present any remarkable symptoms except a hydrocephalus and especially no signs of a precocious puberty. Krabbe<sup>28</sup> was inclined to discredit the suggestion that absence of the pineal gland may occur in man, because he had noticed that when the brain is taken away from the cranium, the gland often becomes detached, the organ remaining hanging by the vena magna galeni. Because of the coexisting congenital abnormalities in the instance recorded in this report, it is not possible to speculate on the possible significance of the apparent absence of the gland as lending support to either of the theories concerning its function. The incident is recorded as a part of a most extraordinary clinicopathologic picture which in the light of present knowledge cannot be explained.

#### THE LYMPHOID TISSUES

The extensive hyperplasia of the lymphoid tissues in the wall of the intestine, the mesenteric lymph nodes and the malpighian bodies

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27. Zandren, Sven: A Contribution to the Study of the Function of the Glandula Pinealis, *Acta med. Scandinav.* **54**:323, 1921.

28. Krabbe, K. H.: The Pineal Gland, Especially in Relation to the Problem on Its Supposed Significance in Sexual Development, *Endocrinology* **7**:379, 1923.

of the spleen is of peculiar interest. Brenner,<sup>29</sup> in a recent paper, accumulated considerable evidence that the site of the essential lesion in Addison's disease is the suprarenal cortex. He brought out the fact that in many cases of Addison's disease, due either to atrophy or to tuberculosis, there is present a status thymicolymphaticus, a generalized hyperplasia of lymph glands or, at times, a lymphoid infiltration of the thyroid gland. Wells<sup>30</sup> pointed out that marked lymphoid infiltration of the thyroid gland is particularly conspicuous in cases of Addison's disease associated with selective destruction of the suprarenal cortex with relatively intact medulla. Jaffe<sup>31</sup> stated that "the newer evidence, including our own work on lymphoid regeneration following suprarenalectomy, supports the view that the lymphoid hyperplasia both in Addison's disease and status lymphaticus is dependent upon insufficiency of the interrenal system (suprarenal cortex)." Marine, Manley and Baumann<sup>32</sup> added further supporting evidence that impaired function of the suprarenal cortex is responsible for thymic and lymphoid hyperplasia. In view of these recent observations, the lymphoid hyperplasia in the case under consideration may or may not be significant, but is of interest, occurring as it does in the presence of a marked increase of both cortical and medullary suprarenal tissue.

#### SUMMARY

The case of a woman who died at the age of 26 years from a cerebral hemorrhage is reported. Clinically her condition had been diagnosed as chronic diffuse nephritis, hypertension, hypogonadism and infantilism. Postmortem examination disclosed a marked hyperplasia of suprarenal tissue associated with pseudohermaphroditismus masculinus externus, marked cardiovascular changes, apparent absence of the pineal gland and an extensive hyperplasia of the lymphoid tissue in the wall of the intestine, the mesenteric lymph nodes and the malpighian bodies of the spleen. The relationship of the suprarenal hyperplasia to the anomalous development of the genital organs and to the hypertensive condition is discussed.

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29. Brenner, O.: Addison's Disease with Atrophy of the Cortex of the Suprarenals, *Quart. J. Med.* **22**:121, 1928.

30. Wells, H. Gideon: Addison's Disease with Selective Destruction of the Suprarenal Cortex, *Arch. Path.* **10**:499, 1930.

31. Jaffe, H. L.: The Influence of the Suprarenal Gland on the Thymus, *J. Exper. Med.* **40**:325, 619 and 753, 1924.

32. Marine, David; Manley, O. T., and Baumann, E. J.: The Influence of Thyroidectomy, Gonadectomy, Suprarenalectomy, and Splenectomy on the Thymus Gland of Rabbits, *J. Exper. Med.* **40**:429, 1924.

# MALIGNANT HEMANGIOMA OF THE LUNG WITH MULTIPLE VISCERAL FOCI

REPORT OF A CASE \*

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At the Babies' Hospital, where over 5,900 autopsies are on record, no primary neoplasm of the lung had been seen until the case reported in this paper was studied in December, 1930. Metastases in the lungs secondary to embryonal adenocarcinoma of the kidney had been observed in four instances. In 1903, Baumann and Bainbridge<sup>1</sup> reported a case of primary sarcoma of the lung at the Great Ormond Street Hospital for Sick Children, where the records from January, 1860, described no other case. Their patient was a girl 3 years and 11 months old. The extreme rarity of primary pulmonary neoplasms in young children and the interesting type of tumor presented by this patient warrant a detailed report.

## REPORT OF CASE<sup>1a</sup>

*History.*—E. B. was admitted on Dec. 3, 1930, at the age of 4 months and 20 days. She died the following day. The family history was negative. The child was born at term, weighing 7 pounds and 10 ounces (3.23 Kg.), and was a "blue baby" for a short time. She was breast-fed for three and a half months, and then was given diluted milk of grade A. Her development was normal, and she sat up at 4 months. When she was from 7 to 8 weeks old, wheezing respiration with attacks of groaning and cyanosis were noted, and were supposed to result from a cold. The attacks were more frequent after eating and lasted from fifteen minutes to four hours. When the child was 4 months old, Nov. 14, 1930, Dr. Kernan made a bronchoscopic examination at the New York Hospital. His report on the examination follows. "The bifurcation and mouths of the main bronchi showed distortion by pressure from the outside. The left bronchus was decidedly more deformed than the right. The mucous membrane was extremely congested. Both bronchi could be seen for a considerable way down their course and no foreign body was seen. Their walls were extremely collapsible and appeared to come in contact when the baby coughed, completely closing the lumen. A very considerable amount of thick mucus material was aspirated. It was thought that there was enlargement, from some cause, of a lymphatic node about the bifurcation of the trachea, producing the distortion of the lumen mentioned. This, with the accumulation of secretion in the bronchi and the collapse of the trachea and the bronchi on coughing, would account for the accumulation of

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\* From the Babies' Hospital and the Pediatric Department of Columbia University College of Physicians and Surgeons.

1. Baumann, E. P., and Bainbridge, F. A.: *Lancet* 1:520, 1903.

1a. Dr. John Kernan consented to the publication of this case.

secretion in the child's lungs and the consequent attacks of dyspnea and cyanosis. Postural drainage is suggested, also atropine in small doses, also x-ray exposure to glands."

A quantity of mucus was removed, after which there was improvement. However, the wheezing returned, and Dr. Kernan sent the child to the Babies' Hospital for a second bronchoscopy. Physical examination showed a well developed and well nourished infant with telangectases on the left side of the face and scalp, a large liver, dulness over the upper lobe of the right lung and hyperresonance over the other lobes, with faint breath sounds, especially over the upper lobe of the right lung. The respirations suggested tracheal obstruction. The mucous membrane of the lower lip was dark purple and shiny; an irregular mottling spread to the lower gum. There were a few moist râles over both lungs, but most of the noises seemed to be transmitted from the throat. The heart was normal. The liver reached the level of the umbilicus, and the spleen was not felt. On admission of the patient the diagnosis was tumor of the lung.

Roentgen examination of the chest was interpreted by Dr. Caffey as showing "the right upper lung field completely obscured by a dense, even shadow with poorly outlined lower borders rather than the usually sharply outlined interlobar fissure. The trachea was displaced markedly to the left. The left lung field appeared normal." Dr. Kernan's note on the result of the second bronchoscopy reads: "Displacement of the carina and intensely inflamed and swollen bronchial mucosa especially on the left side. It is thought there is still pressure on the trachea and bronchi from large glands accompanied by bronchitis and noticeable collapse of the trachea and bronchi on expiration, explaining the emphysema." Following the bronchoscopy the child had great difficulty in breathing, and respirations ceased several times, being started again by vigorous methods of artificial respiration, administration of epinephrine hydrochloride, etc. The child ceased breathing after repeated attacks of this type in which there was respiratory failure. Dr. W. R. Williams permitted me to examine the x-ray films taken from Oct. 17 to Nov. 26, 1930, at the New York Hospital. At that time the shadow in the upper lobe of the right lung was evident. On November 7, at the New York Hospital, the red cells numbered 3,320,000 and the leukocytes 7,560, with polymorphonuclears 51 per cent and lymphocytes 49½ per cent. The hemoglobin was 60 per cent.

*Autopsy.*—Autopsy was performed by Dr. Beryl H. Paige. The body was that of a normally developed, moderately well nourished, white female infant. On the left cheek and neck were dilated vessels, and in the lower lip were two small hemangiomas, one on each side of the midline. A small umbilical hernia was present. Permission was given for a thoracic incision only.

The left pleural cavity was free from fluid and adhesions. In the right there were dense fibrous adhesions between the parietal pleura and the visceral pleura covering the upper lobe posteriorly. The heart, esophagus, thyroid gland, pancreas and kidneys were normal. The thymus gland weighed only 6 Gm. The larynx contained much mucus, and the wall showed injection in a narrow zone 5 mm. wide. The mucosa in the lower portion of the trachea showed slight injection, but the wall was free from exudate. In the right main bronchus there was a large amount of muroid material and slight injection was noted in the proximal 5 mm. of the mucosal wall, while immediately above the division several blackish-red areas (hemangiomas), from 2 to 3 mm. in size, lay in the mucosa. In the left main bronchus there was also some mucus. The proximal 1 cm. of the mucosa was bright red, and was the seat of an angioma 1 cm. in length (fig. 1a), extending around almost the entire caliber of the bronchus.

The upper lobe of the right lung was voluminous, measuring 7.5 cm. in its greatest length. On the posterior surface were numerous fibrous adhesions. On the anterior surface was an incomplete accessory fissure, lying from 0.5 to 1.5 cm. above the lower anterior margin of the lobe. The posterior third of the upper lobe was deep red and fleshy in consistency, but not firm. On the surface the lobules were clearly marked. A vertical zone, 2.5 cm. wide, anterior to this area was moderately emphysematous and pale, and the remainder of the upper lobe was deeper red, but air-containing. The lower and middle lobes were bright pink and well aerated. On section, the cut surface of the posterior part of the upper lobe was sharply differentiated into lobules from 1 to 3 cm. in diameter, (fig. 1*b*). The tissue was dark red and fleshy. In the centers of many of the lobules was a pale gray area about 5 mm. in diameter, and these paler areas surrounded small branches of the bronchial tree. The cut surface of the lower lobe was bright

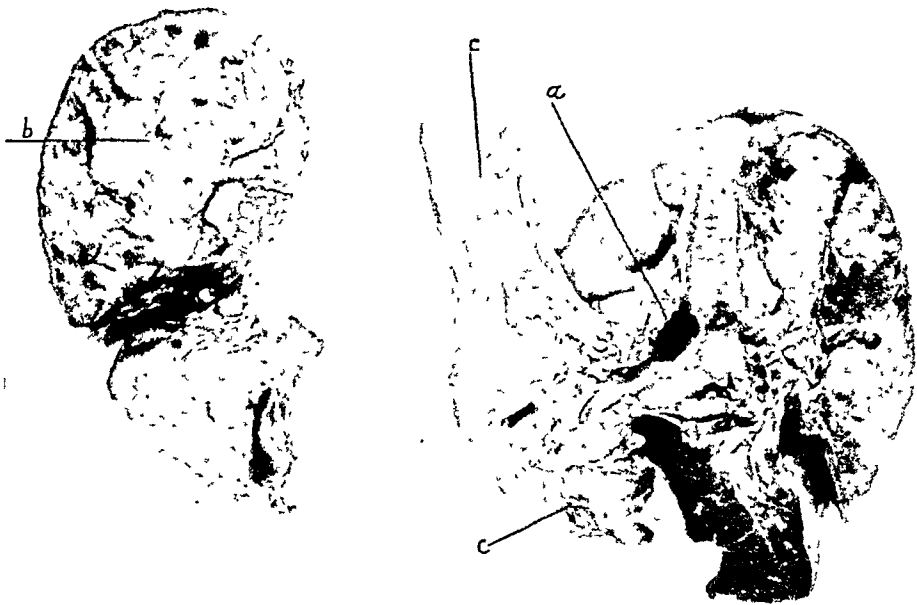


Fig. 1.—At the left is the cut surface of the right lung, showing the lobular outlines and the light central areas of the new growth in the upper lobe (*b*). The left lung, shown at the right, is aerated (*c*). The left main bronchus shows a hemangioma (*a*).

pink and aerated. The left lung was bright pink and well aerated. On the mediastinal margin of the upper lobe near the apex was a lobule over which the pleura showed injection. This lung was normal on section. The tracheo-bronchial lymph glands were enlarged, the longest being 2 cm. in diameter. They were bright red, soft and discreet.

The spleen weighed 24 Gm. It was deep purplish red, and on section the malpighian bodies were from 1 to 2 mm. in diameter and numerous. The liver weighed 256 Gm. The capsule was normal. The organ was pale, reddish brown and moderately fatty. The gallbladder was normal. In the coils of the small intestine were irregular, deep-purplish areas, where the mucosa showed injection, and the Peyer's patches and the follicles were hyperemic. A mesenteric lymph node was pale gray. The lymph nodes around the pancreas

were deep red, discreet and less than 1 cm. in width. The suprarenal glands were of average size. The cortices were pale yellow, and the central zone appeared as a fine, reddish-brown line. Adherent to the hilus of the right gland was a circumscribed flattened mass of deep-red tissue resembling the lymph glands around the pancreas. One ovary appeared normal, and the other contained several cysts.

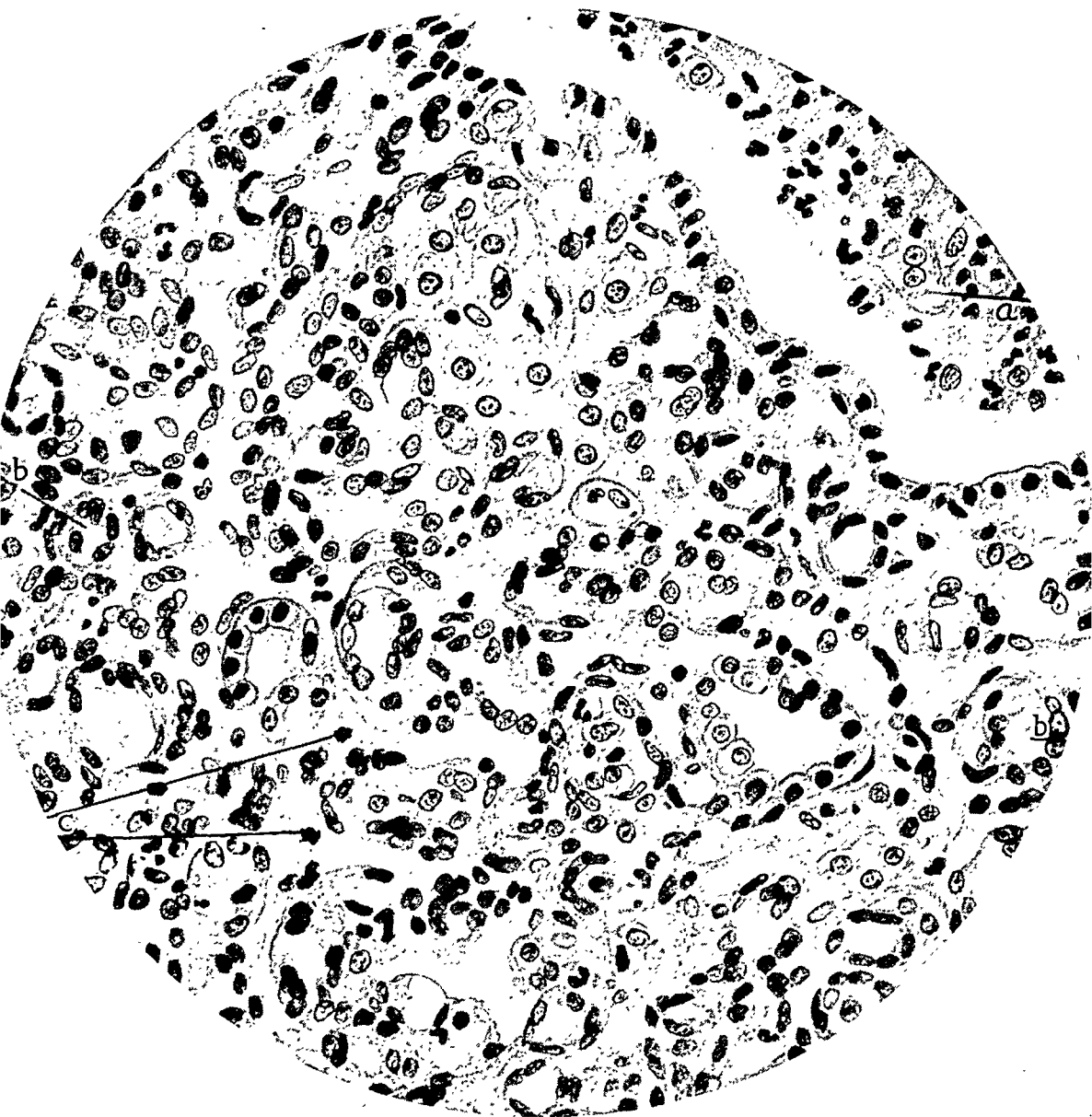


Fig. 2.—A section of the neoplasm in the upper lobe of the right lung, showing bronchiole containing purulent exudate (*a*), vascular channels lined by endothelial cells (*b*), and mitoses in endothelial cells (*c*).

*Microscopic Examination.*—It would hardly have been possible to identify a section of the upper lobe of the right lung as lung if it had not been for the bronchioles filled with purulent exudate (fig. 2*a*). Around them were no normal alveoli, only masses of blood vessels the lining of which was a single layer of endothelial cells, cuboidal in many places, flattened in others. Their nuclei

were vesicular and hyperchromatic, and a few mitoses were seen. Such vessels contained red blood cells. Between them there was no stroma, merely a basement membrane that stained black with the Weigert-van Gieson stain. These vessels or channels varied in size, but they were capillaries (fig. 2*b*). There were fields in which the growth was more solid and less vascular, the cellular buds not having become canalized. In places the pulmonary alveoli and bronchioles had been compressed to mere slits; in others they were less compressed and were lined with cuboidal (embryonal type) epithelium, but in most fields of the solid tumor no trace of them remained. Mitoses were most common in the solid fields (fig. 2*c*). The veins and arteries in the connective tissue septums between the lobules were filled with red cells. A section taken from the margin, or youngest

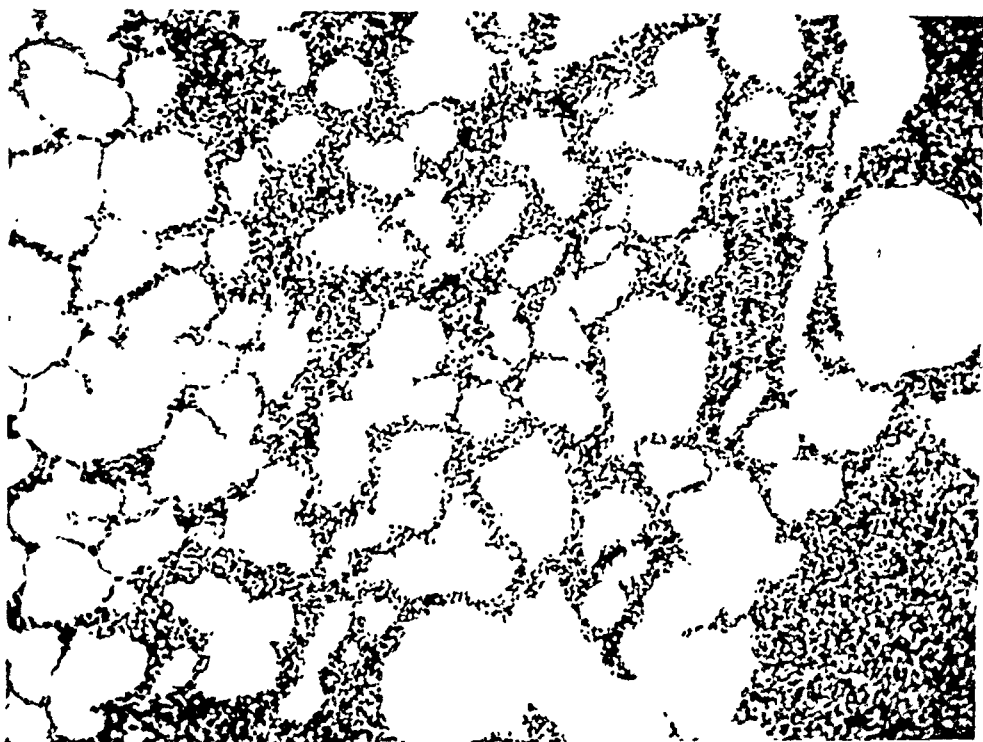


Fig. 3.—A section of the upper lobe of the right lung, showing progressive invasion through the alveolar walls at the edge of the neoplasm.

part, of the tumor showed large areas of cellular growth with little tendency to the formation of blood channels, but much more variation in the size and shape of the nuclei. Many were large, irregular and hyperchromatic. The tumor was growing or progressing through the alveolar walls, thickening them and compressing the alveoli (fig. 3). The invasive malignancy of the new growth was marked in the lung.

The mucosa of the right bronchus was widened, and the newly formed capillaries, filled with red cells, extended almost to the covering epithelium. There were also areas of hemangioma in the mucosa, between the mucous glands, and these extended through the muscularis mucosae into the submucosa and into the connective tissue between the cartilage plates, then into the perichondrium almost to the muscularis, which was not invaded. The newly formed vessels were lined with cuboidal endothelium and contained red cells. There were also solid buds of endothelial cells, not as yet canalized. It seemed plain that the bronchial wall

became invaded by the neoplasm from the parenchyma of the lung, the lobules of angioma becoming largest in the mucosa, where the loose-meshed tissue offered less resistance to proliferation.

In the small intestine, the covering epithelium of the villi was intact, and some villi contained congested capillaries. The mucosa proper contained areas made of small vessels filled with red cells and lined with cuboidal endothelial cells. These vessels or channels were closely packed, and extended through the muscularis mucosae into the submucosa, widening and reddening it. But they stopped at the margin of the circular muscle coat, which was free, as were the lymph follicles in the submucosa. The areas of hemangioma could be plainly distinguished from the uninvolved mucosa, and while solid buds invaded both villi and submucosa,



Fig. 4.—A section of the suprarenal gland showing a cavernous type of hemangioma in the medullary zone.

there were places where the outline of the neoplasm was distinct. In the surrounding fat were lobules of tumor tissue.

The cortical cells of the suprarenal gland contained lipoid, and the reticular layer cells were degenerating. In the medullary zone of the suprarenal gland numerous large and small endothelium-lined channels contained erythrocytes and leukocytes. The channels were separated by small strands of connective tissue, their lining cells were flattened, and the picture differed from the rest of the growth because it was distinctly cavernous in type (fig. 4). External to the suprarenal gland were numerous circumscribed lobules of tumor tissue invading and replacing fat lobules, again illustrating the progressive, invasive growth of the neoplasm. A sympathetic ganglion next to the suprarenal gland showed angiomatous invasion.

A few lobules of the pancreas showed hemangiomatous areas, not sharply circumscribed but extending irregularly into the pancreatic lobules. A mass of tissue



taken from the prevertebral area of the upper part of the abdomen showed an uninvaded lymph node and hemangioma apparently replacing fat, as well as uninvaded lobules of adipose tissue. A tracheobronchial lymph gland was free from tumor, but lobules of angioma were replacing fat lobules. The freedom of both thoracic and abdominal lymph nodes from invasion by the tumor was striking. A nodule from the region of the pancreas showed, in addition to pancreas and an accessory spleen, numerous circumscribed lobules of angiomatous tissue and some normal lobules of fat. In the accessory spleen the lymphoid follicles were hyperplastic, the sinuses contained many red blood cells and polymorphonuclears, the capsule was intact, and no evidence of invasion by the tumor was found.

The malpighian bodies of the spleen were large. No tumor was present, nor was there any in the liver or in the kidney. It is to be regretted that no specimen from the skin (lip, cheek, scalp) could be examined, but the restrictions of the autopsy made that impossible.

*Diagnosis.*—The anatomic diagnosis was: malignant hemangioma of the right lung, bronchi, small intestine, pancreas, suprarenal gland, adipose tissue, abdominal sympathetic ganglion, scalp and lip; fibrous pleural adhesions; acute bronchitis; fatty liver, and cysts of the ovaries.

#### COMMENT

The gross appearance of the tumor in the upper lobe of the right lung suggested sarcoma. The whole mass was firm, but not hard, shading imperceptibly into aerated pulmonary substance. Microscopically, the lighter areas were those in which the tumor was young, with fewer new vascular channels filled with blood, but with many solid cell masses and compressed alveoli. The extension of the growth into uninvolved lung is well shown in figure 3, which illustrates the proliferating endothelial cells spreading as solid buds along the alveolar walls and into the dilated alveolar ducts as solid masses, sometimes knobbed. In the fat around the right suprarenal gland, the advancing growth showed some similar projections covered by a single layer of endothelial cells. In the lung there were areas in which the cells were arranged in smaller or larger groups or sheets with no canalization, but hardly primitive enough to suggest syncytial masses. These cells had an acidophil cytoplasm, which was granular, and a deeply staining nucleus with occasional mitoses. Close by were narrow blood channels containing red cells, the wall made of a single layer of endothelial cells, which were often cuboidal. These vessels had no stroma between them and compressed the adjacent alveoli so that many of them were mere slits with lining cells that had returned to the embryonal cuboidal type. The growth was malignant and invasive as seen in all the foci, especially the parenchyma of the lung, the intestine, the fat tissue and the sympathetic ganglion. Only in the suprarenal medulla did the tumor assume the cavernous type, while in the adjacent fat it was again formed of capillaries only.

In the literature reports of cases of primary sarcoma of the lung in children are few, and variously designated. Thus Chiari<sup>2</sup> reported a spindle cell sarcoma occurring in the right upper pulmonary lobe of a 14 year old girl. Round cell sarcomas have been described by Hagenbach,<sup>3</sup> Björnsten,<sup>4</sup> Lehdorf<sup>5</sup> and Peters;<sup>6</sup> a lymphosarcoma by Gannon,<sup>7</sup> and a polymorphous cell sarcoma by Acuna and his associates.<sup>8</sup> Rosenblum and Gasul's<sup>9</sup> patient was a girl 29 months old with a large sarcoma of the right lung, but no microscopic details are given. None of these descriptions fits this case, which is so evidently of vascular origin, the type cell being the endothelial cell, and the microscopic picture that of malignant hemangioma. In 1928, Wright<sup>10</sup> collected seven cases of hemangiomatous tumors that formed metastases, and added one of his own. All the tumors occurred in adults, and none was primary in the lung. Four were histologically benign (Borrmann,<sup>11</sup> Ewing,<sup>12</sup> Homans,<sup>13</sup> Shennan<sup>14</sup>), and four were histologically malignant (Langhans,<sup>15</sup> Theile,<sup>16</sup> Jores,<sup>17</sup> Wright<sup>10</sup>). The recent article by Schlopsnies<sup>18</sup> details another case in an adult, the growth involving the spleen, liver and bone marrow. Theile<sup>16</sup> and Jores<sup>17</sup> classified their cases as sarcomatous angioma, and Lubarsch<sup>19</sup> included them both in the group of angioblastic sarcomas or sarcomatous angiomas, for which Borst<sup>20</sup> assumed an origin from endothelial cells that proliferate in the form of buds (embryonal vessels), which in places may become canalized, thus differentiating into vessels the lining cells of which are numerous and

2. Chiari, H.: *Anz. d. k. k. Gesellsch. d. Aerzte in Wien*, 1877, no. 6, p. 29.

3. Hagenbach, cited by Boschowsky: *Frankfurt. Ztschr. f. Path.* **9**:239, 1911-1912.

4. Björnsten, cited by Boschowsky (footnote 3).

5. Lehdorf, H.: *Wien. med. Wchnschr.* **59**:1774, 1909.

6. Peters, C. A.: *M. Clin. North America* **7**:1823, 1924.

7. Gannon, N. D.: *Pennsylvania M. J.* **32**:574, 1928-1929.

8. Acuna, M.; Wincour, P., and Orosco, G. P.: *Arch. latino-am. de pediat.* **23**:605, 1929.

9. Rosenblum, P., and Gasul, A.: *Arch. Pediat.* **48**:63, 1931.

10. Wright, A. W.: *Am. J. Path.* **4**:507, 1928.

11. Borrmann, R.: *Beitr. z. path. Anat. u. z. allg. Path.* **40**:372, 1907.

12. Ewing, J.: *Neoplastic Diseases*, ed. 3, Philadelphia, W. B. Saunders Company, 1928.

13. Homans, J.: *Ann. Surg.* **25**:732, 1897.

14. Shennan, T.: *J. Path. & Bact.* **19**:139, 1915.

15. Langhans, T.: *Virchows Arch. f. path. Anat.* **75**:273, 1879.

16. Theile: *Virchows Arch. f. path. Anat.* **178**:296, 1904.

17. Jores, L.: *Zentralbl. f. path. Anat.* **19**:662, 1908.

18. Schlopsnies, W.: *Virchows Arch. f. path. Anat.* **85**:274, 1929-1930.

19. Lubarsch, O.: *Pathologische Anatomie der Milz*, in Henke and Lubarsch: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1927, vol. 1, p. 2.

20. Borst, M.: *Pathologische Histologie*, Leipzig, F. C. W. Vogel, 1926.

may appear in several layers. The cells giving rise to this type of neoplasm are angioblasts in their primitive state, endothelial cells in the next stage of their differentiation and potential precursors of sarcoma cells and of blood cells. Wright<sup>10</sup> believed that the tumor that he reported grew from cells less primitive than angioblasts, that is, endothelial cells, but no longer characteristic endothelia, because they had undergone neoplastic change. In the case here reported angioblasts were not present, but the origin from endothelial cells was evident, and the sarcomatous areas were less marked than in those described by Theile<sup>16</sup> and by Schlopsnies.<sup>18</sup> The latter also remarked the lack of involvement of the lymph nodes, and in reviewing the cases belonging in this group of neoplasms, found that the liver was the primary seat in about twenty cases, the spleen in eleven or twelve, the bone marrow in one, the thymus gland in ten and the skin only once. The lung, as the principal focus, has not hitherto been noted.

The case here reported is apparently the first in which the principal or largest mass was in the lung, with smaller foci in other organs. It would be difficult to say which was the primary growth, but the lung was undoubtedly the organ most involved. In this connection Lubarsch<sup>19</sup> raised the question whether in these neoplasms there was a primary growth with metastases, or whether there were not rather independent growths or multiple foci of origin, an "angiomatosis multiplex or even universalis." In the present case multiple foci seem best to explain the condition, for while the foci differed in size, they were of the same age, that in the lung evidently being the most rapidly growing, although the manner of invasion of the perisuprarenal gland and prevertebral fat lobules was as active.

The malignancy of the growth is shown by its local invasiveness in every focus, and by the mitoses in the neoplastic cells present in these foci. The spidering of vessels in the face was noted when the patient was admitted to the New York Hospital, and a month later at the Babies' Hospital an angioma of the lip, spreading to the gum, was seen. This would seem to argue progressive involvement of the skin.

Only a few cases of multiple hemangiomas occurring in children have been recorded. Stamm<sup>21</sup> described one in a 4 months old infant which involved the skin, muscles, both lungs, right vocal cord, ovaries, small intestine and cerebral cortex, and diagnosed it as angioma simplex. Ramdohr<sup>22</sup> detailed the case of a new-born infant who died of hemorrhage from a tumor on the inferior maxilla and had multiple nodules of angiosarcoma in the skin, lungs and kidneys. The lungs were not

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21. Stamm, C.: Beiträge zur Lehre die Gefässgeschwülsten, Dissertation, Göttingen, 1891.

22. Ramdohr, M.: Virchows Arch. f. path. Anat. **73**:459, 1878.

involved in the cases occurring in children reported by Brüchanow,<sup>23</sup> Ernst<sup>24</sup> and von Falkowski;<sup>25</sup> von Falkowski's patient, a boy  $2\frac{3}{4}$  months old, had angiomas of the skin, spleen and liver, which he classified as a peculiar form of mesenchymal benign hamartoma, the result of a systemic disease of the embryonal mesenchyme, a tissue maldevelopment. In Ernst's<sup>24</sup> patient, 2 months and 20 days old, the liver, spleen and skin were the seat of nodules diagnosed as congenital angioma simplex. Brüchanow's<sup>23</sup> patient, 15 weeks old, showed multiple hemangiomas of the skin, the liver and the periosteum of two ribs. Böckelmann's<sup>26</sup> patient, 15 months old, underwent splenectomy for an angiosarcoma of the spleen, and de Haan<sup>27</sup> reported an alveolar angiosarcoma of the liver without metastases in a baby 4 months old. It is apparent that the lungs are but infrequently involved in neoplasms of the group of malignant hemangiomas.

#### SUMMARY

A case of multiple malignant hemangiomas in an infant, involving the lung, skin, bronchi, pancreas, intestine, suprarenal gland, prevertebral fat and sympathetic ganglion, is described. The principal and largest mass was in the upper lobe of the right lung, a condition not noted in any other reported case. The lymph nodes were not involved in the neoplastic process.

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23. Brüchanow, N.: *Ztschr. f. Heilk.* **20**:131, 1899.

24. Ernst, P.: *Verhandl. d. deutsch. path. Gesellsch.* **15**:232, 1912.

25. von Falkowski, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **57**:385, 1913.

26. Böckelmann: *Ueber ein Angiom der Milz*; *Inaug. Diss.*, Greifswald, 1906.

27. de Haan, J.: *Beitr. z. path. Anat. u. z. allg. Path.* **34**:215, 1903

# EXPERIMENTAL SCARLATINAL NEPHRITIS IN THE DOG \*

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NEW ORLEANS

The present views on the sequence of the early anatomic changes that underlie certain clinical types of nephritis are largely conjecture. This is due, in part, at least, to the fact that the renal function is so complex and the possible lesions so varied that an accurate correlation of the urinary observations and the renal changes is prevented. It is generally held that abnormalities in the urine as determined by microscopic and chemical analysis are only presumptive evidence of nephritis, since these may be present without demonstrable renal lesions. Even when the urinary observations point to renal changes, they are not a reliable criterion of the location, extent and degree of these changes, because the exact relations between renal structures and their functional activities are imperfectly understood. Therefore, albumin, casts and blood in the urine offer little information as to whether there is a renal structural change or merely a functional disturbance.

My purpose in this paper is to report the results of experiments on animals which were instituted in order to determine as far as possible the relation of the renal structural changes, and to trace their development in correlation with abnormal urinary findings. Experimental nephritis was induced in dogs with (1) living culture, (2) killed culture and (3) the filtered in vivo prepared toxic product of *Streptococcus scarlatinae*.

## EXPERIMENTS

Since nephritis frequently occurs spontaneously in the dog, I used only young animals the urine of which over a period of from ten days to two weeks was free from albumin, casts and other abnormalities, and the renal function of which, as determined by the phenolsulphonphthalein test, was within the normal limits. During the period of observation the animals were kept in metabolism cages to facilitate the collection of urine. Catheterized specimens were not employed; hence the figures in the tables for the volumes of urine are averages only, which were obtained by dividing the total volume of urine of seven days

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by the number of days in the period. Daily examinations of urine were made over a period of from one to three weeks after the administration of the nephrotoxic agent. In the case of dogs that survived several months, the examinations of the urine were made at weekly intervals after the first three weeks. The phenolsulphonphthalein test was repeatedly carried out on all dogs at approximately weekly intervals.

Fourteen young, healthy dogs were employed in the experiments. Nephritis was induced by intraperitoneal and intravenous injections of "living" and "killed" cultures of scarlatinal streptococci and of a "lysate" of these streptococci (prepared after the method of Duval and Hibbard<sup>1</sup>). The dosage of "killed" culture was the entire forty-eight hour surface growth of twelve blood agar slants suspended in from 15 to 30 cc. of sterile physiologic solution of sodium chloride; while the dose of the "lysate" was from 15 to 30 cc. of sterile Berkefeld "N" filtered peritoneal fluid from an immune rabbit that had previously received intraperitoneally the forty-eight hour streptococcal surface growth of twelve blood agar slants. The dosage of "living" culture employed was the forty-eight hour surface growth of three blood agar slants suspended in 10 cc. of sterile water.

The following protocols are representative of the results obtained:

*Dog 1.*—Dog 1 was a female weighing 5 Kg. During a preliminary period of observation of one month daily samples of urine were found free from abnormal constituents. The animal was given an intraperitoneal injection of 30 cc. of lysate of scarlatinal streptococci prepared *in vivo*. One hour later the dog vomited and appeared greatly prostrated. On the following day the animal was better, but refused food. Three days after the first injection, the dog ate again and appeared well. Two weeks after the injection albumin appeared in the urine and continued to appear for the remaining period of the experiment. Three weeks after the first injection a second injection of 15 cc. of lysate was given intraperitoneally. Two weeks later a third intraperitoneal injection of 15 cc. of lysate was given. After each injection the dog vomited and appeared very ill for two days. The second and third injections apparently caused greater quantities of albumin to appear in the urine, and after the third injection blood and casts, both fine and granular, were also noted. The dog was found dead in its cage one week after the third injection or approximately two months after the first injection of lysate.

At autopsy there was the usual picture of a severe toxemia in the spleen, heart, liver and kidneys. The kidneys in particular were markedly swollen and generally dark red. On section the cortex presented conglomerate and discrete pinhead-sized and smaller, yellowish-white areas. The glomeruli were discerned as dark red, elevated dots, resembling in many instances petechiae. Because of these hemorrhagic spots near the capsule, the cortical surface of the kidney presented the classic "turkey-egg" spotting.

Microscopically, the outstanding lesions were in the glomeruli, and varied from simple hyperemia of the capillary tufts to rupture and actual hemorrhage into the capsular space.

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1. Duval, C. W., and Hibbard, R. J.: *J. Exper. Med.* **44**:567, 1926.

*Dog 3.*—Dog 3 was a female weighing 11.1 Kg. During the preliminary period of observation (eight days) the urine remained normal. On January 24, the first intravenous injection of 20 cc. of a suspension of killed scarlatinal streptococci was made. Similar amounts of the killed culture were injected sixteen and twenty-six days later. Albumin, blood and a trace of bile appeared in the urine two days

TABLE 1.—*Urinary Findings in Nonfatal Experimental Nephritis Induced in Dog 3 with Three Intravenous Injections of Killed Scarlatinal Streptococci*

Date	Volume of Urine Excreted in 24 Hours, Cc.	Amount of Killed Culture Injected Intravenously, Cc.	Albumin	Blood	Bile	Casts			Phenolsulphon-phthalein Excreted, per Cent	Comment
						Hyaline	Fine Granular	Coarse Granular		
1/24	363	20	0	0	0	0	0	0	67.4	First injection
1/25	270	..	0	0	0	0	0	0	....	
1/26	280	..	+	+	0	0	0	0	....	First clinical evidence of nephritis
2/ 8	350	..	+	0	0	0	0	0	....	
2/ 9	365	20	+	0	0	0	0	0	....	Second injection
2/11	205	..	++	+	0	0	0	0	....	Increased albumin; blood
2/13	500	..	++	+++	+	0	0	0	....	Marked hematuria; bile
2/14	220	..	++	++	0	0	0	0	52.0	
2/15	210	..	+	+	0	0	0	0	....	
2/16	200	..	+	+	0	0	0	0	....	
2/17	215	..	+	0	0	0	0	0	....	Disappearance of blood
2/18	215	..	+	0	0	0	0	0	....	
2/19	520	20	+	0	0	0	0	0	....	Third injection
2/21	180	..	+	0	0	0	0	0	....	
2/22	220	..	+++	++	+	++	++	++	46.7	First appearance of casts
2/23	275	..	+++	+	+	++	++	++	....	
2/27	275	..	+++	+	+	+	+	+	....	Decreased excretion of dye
2/28	90	..	++	0	+	0	0	0	....	Oliguria marked
3/ 2	230	..	+++	0	+	0	0	0	....	
3/ 4	380	..	+	0	0	0	0	0	....	
3/ 5	240	..	+	+	+	0	0	0	....	
3/16	240	..	+	+	+	0	0	0	....	
3/23	130	..	0	0	0	0	0	0	59.3	Diminished excretion of urine
3/24	300	..	0	0	0	0	0	0	....	
3/31	300	..	0	0	0	0	0	0	....	
4/ 5	435	..	0	0	0	+	+	+	....	Reappearance of casts
4/ 8	350	..	+	0	+	+	+	+	....	
4/15	400	..	0	0	0	+	+	+	....	
4/22	250	..	+	0	+	+	+	+	....	Persistence of albumin and casts
5/ 3	200	..	+	0	+	+	+	+	41.2	
5/11	250	..	+	0	+	+	+	+	....	Evidence of chronic nephritis
5/18	Dog was killed. Autopsy revealed acute and chronic nephritis									

after the first injection (see table 1). In addition to albumin and blood, hyaline and granular casts appeared in the urine three days after the third intraperitoneal injection of "killed" culture. These pathologic substances were observed in the urine at every subsequent examination over a period of three months, though the dog was fat and appeared in good health. The animal was killed five months after the first inoculation. The changes observed at autopsy were acute glomerulonephritis, tubular nephritis and interstitial infiltration with lymphocytes. There was no increase in the connective tissue.

*Dog 4.*—Dog 4 was a female weighing 6.7 Kg. This animal was under daily observation for twelve days prior to the experiment. During this time the urine and the renal function were normal. On January 24, 15 cc. of streptococcal lysate





was injected intraperitoneally. Subsequent injections of lysate in the same dosage were made on February 9 and 19. After each injection the dog vomited and refused food for twenty-four hours. After the third injection diarrhea developed, and the animal was found dead in its cage five days later. Albumin was first noted in the urine six days after the first injection and was present at every subsequent examination. Bile, large numbers of hyaline and granular casts and blood appeared in the urine after the third injection. Three days later the animal died, and at autopsy the kidneys showed diffuse hemorrhagic glomerulonephritis.

*Dog 11.*—Dog 11 was a female weighing 6.1 Kg. The preliminary period of observation lasted for two weeks, during which daily tests made of the urine revealed no abnormalities. The phenolsulphonphthalein test at this time showed 72 per cent excretion of the dye. On February 22, 10 cc. of streptococcal lysate was injected intraperitoneally. Vomiting occurred immediately. This animal

TABLE 4.—*Urinary Findings in Fatal Experimental Nephritis Induced in Dog 14 with Single Intravenous Injection of "Lysate" of Scarletinal Streptococci*

Date	Volume of Urine Excreted in 24 Hours, Cc.	Amount of "Lysate" Injected Intravenously, Cc.	Albumin	Blood	Bile	Casts			Phenolsulphonphthalein Excreted, per Cent	Comment
						Hyaline	Fine Granular	Coarse Granular		
4/ 6	500	12	0	0	0	0	0	0	84	First injection; animal vomited
4/ 7	400	..	++	+	+	++	++	++	..	Marked evidence of nephritis
4/ 8	340	..	++	+	+	+	+	+	..	Animal very sick
4/ 9	500	..	+	+	+	+	+	0	..	Animal improved
4/14	200	..	+	0	++	+	0	+	60	Excretion of dye diminished
4/22	210	..	+	+	++	+	+	+	..	Animal not eating
4/24	300	..	++	++	+	++	++	+	..	Severe diarrhea
4/25	200	..	++	+	++	++	++	++	..	Very weak and sick
4/27	Dog was found dead in cage. Autopsy revealed acute hemorrhagic glomerulonephritis									

received only one injection. Albumin, bile, blood, hyaline and coarsely granular casts appeared in the urine forty-eight hours after the injection. Albumin and bile were excreted continuously over a period of four months, until the animal was killed.

Autopsy revealed acute glomerulonephritis and early interstitial leukocytic infiltration as the outstanding renal changes.

*Dog 13.*—Dog 13 was a male weighing 6.7 Kg. During the preliminary observation (ten days), all samples of urine were free from pathologic constituents. On April 28, 15 cc. of streptococcal lysate was injected intravenously. On the following day albumin and hyaline and granular casts were found in the urine. Thereafter albumin was present in the urine to the end of the experiment. A second injection of 15 cc. of lysate was given intravenously on May 3, five days after the first injection, which failed to cause vomiting or sickness. After May 3 traces of bile and blood were excreted and casts and pus appeared occasionally until June 12, when the dog was killed. The autopsy revealed albuminous and fatty degeneration of the liver and heart and acute glomerulonephritis.

*Dog 5.*—Dog 5 was a male weighing 6.6 Kg. During the preliminary period of observation (twelve days) the urine was normal. On February 19, 20 cc. of a suspension of living scarlatinal streptococci was injected intraperitoneally. Vomit-

ing and diarrhea occurred one hour later, and the animal appeared ill. The following day the dog was comatose and died. The urine showed great quantities of albumin.

Autopsy disclosed acute glomerulonephritis and a fatty heart and liver.

*Dog 7.*—Dog 7 was a female weighing 6.8 Kg. The animal was under observation for two weeks prior to experimentation; during this time the urine was normal. Lysate to the amount of 15 cc. was administered by intravenous injection on March 16. The dog vomited shortly after the injection. The following day it appeared normal. On March 18, albumin, hyaline and granular casts and bile appeared in the urine. On March 23, the excretion of phenolsulphonphthalein in two hours was 64 per cent. One month later this test showed 78 per cent excretion of the dye, which is normal, and there were no abnormalities in the urine. The animal was killed, and autopsy revealed acute glomerular and slight chronic interstitial nephritis.

*Dog 14.*—Dog 14 was a male weighing 13.6 Kg. Prior to the experiment the urine was examined daily for twelve days and found to be normal. On April 6, 12 cc. of lysate was injected intravenously. The animal vomited following the injection. On the second day after the injection, albumin, hyaline and granular casts, blood and bile appeared in the urine. These persisted in the urine to the end of the experiment. The animal was sick throughout the period of observation, and died twenty-one days after the injection of lysate.

Autopsy showed acute hemorrhagic nephritis, fatty liver and hypertrophy and dilatation of the heart.

#### THE RENAL CHANGES IN STRUCTURE

The acute renal lesion of the experimental scarlatinal nephritis in these dogs was characterized macroscopically by an increase in the size of the kidney, by swelling of the glomeruli, which projected above the cut surface as dark red dots, and by pinhead and smaller, discrete, yellowish-white lineations in the cortical substance and in some instances by a sprinkling throughout the organ of minute hemorrhages (turkey-egg spotting). The gross appearance of the kidney in chronic nephritis in these dogs was that of an organ of normal or slightly under-normal size, in which the consistence was increased and the color pale reddish brown. There were also numerous small retention cysts and cicatricial depressions scattered through the cortical substance.

The earliest microscopic change was an intense engorgement of the capillaries of the glomeruli with often densely packed erythrocytes that appeared as though the hemoglobin had been dissolved out. In many of the capillary tufts the loops were enormously distended with what appeared to be erythrocytic thrombi. Other capillaries were distended by masses of eosin-staining material, which was homogeneous and which apparently was formed from the fused and destroyed red blood cells. Many of these hyaline-thrombosed capillary loops were glued to the wall of Bowman's capsule. In the case of some dead capillary loops, new connective tissue partially or completely cicatrized them. In other glomeruli where the capillaries were not blocked by thrombi, the lumina contained large numbers of mononucleated cells. The nature of these

cells was difficult to determine, since they appeared to be outside of the capillaries in the capsular space; however, in no instance were they of the neutrophil variety. I am inclined to regard them as endothelial cells because of their manner of staining and because of the character of the nucleus, which was definitely vesicular and surrounded with considerable basic-staining cytoplasm. In still other glomeruli extensive hemorrhage was noted, which, in some instances, could be readily traced into the corresponding tubule. Sometimes the hemorrhage into the subcapsular space was so large as to misplace or crowd out the capillary tuft. When blood escaped into the subcapsular space, the red cells fused into a homogeneous, pink-staining mass which often became attached to the capsule. In these masses there commonly occurred connective tissue and an invasion by epithelial cells producing the so-called "crescent." The replacement of the hyalinized capillaries by fibrous tissue produced complete or partial glomerular sclerosis.

The tubular epithelium was not affected until late in the glomerular process; then it became swollen, granular and filled with fat droplets; especially was this the case with the epithelium of the convoluted portion of the tubule. In places the epithelium had desquamated and the tubule had atrophied, and eventually in those instances in which the corresponding glomerulus was destroyed the tubule degenerated. All kinds of casts were demonstrated. A significant feature was the absence of any acute inflammatory lesion in and about the involved tubules.

When a dog had received several injections of streptococcal lysate, and had been allowed to live for six months or longer, and previous examinations of the urine had shown the existence of an acute glomerular nephritis, microscopic study of the kidneys revealed considerable change of a reparatory character in the interstitial connective tissue. In these kidneys there was noted an increase of fibrous tissue in the areas where the greatest damage had occurred to the glomeruli. In such areas of connective tissue many of the tubules were atrophic, and the glomeruli were in various stages of retrogression, some being actually necrosed. Some convoluted parts of tubules in the affected areas appeared larger and others smaller than normal; the larger ones in some instances were even cystic, owing to the mechanical interference with the passage of urine caused apparently by the contraction of the adjacent newly formed connective tissue.

The animals that received the living culture of streptococci showed at autopsy as the outstanding renal lesion focal and diffuse lymphocytic interstitial infiltration. The kidneys also showed a moderate degree of glomerular, but no tubular, change. Few if any neutrophils occurred in the stromal lesion. Streptococci were demonstrable in the stained renal sections and were recoverable in pure culture from the fresh tissue.

## CORRELATION OF URINARY OBSERVATIONS AND RENAL DISTURBANCES

The sequence and relation of the renal changes in correlation with the functional disturbances as expressed by abnormal urinary constituents were studied from the commencement of the acute renal lesion until chronic nephritis was well established.

The appearance of albumin and casts in the urine was constant in all the dogs given injections. In most cases albumin and casts occurred within twenty-four hours after the injection of the nephrotoxic agent, while in others these abnormalities were found only after from two to three days (see table 1). In addition to albumin and casts, the majority of the dogs showed blood and traces of bile in the urine, although usually these substances did not appear before the third day following the injection (see table 2).

Four of the acutely nephritic dogs were allowed to live for six months or longer. These animals in two or three months after the third and last injection of the streptococcal nephrotoxin apparently returned to a normal renal function as indicated by the total absence of abnormal constituents in the urine. However, at autopsy there was definite gross evidence of chronic renal changes, which was confirmed by microscopic study.

It is particularly noteworthy that the urinary findings from day to day were a fairly reliable index to the anatomic changes in the kidneys. Blood in the urine invariably meant glomerular hemorrhage, while fine, granular casts foretold serious retrograde changes in the epithelium of the convoluted tubules, which seemed to be dependent on, but always secondary to, lesions of an obstructive character in the glomerular tufts. Here it is of interest to mention that the primary glomerular lesions followed by secondary tubular changes is the reverse of the sequence induced in dogs with uranium nitrate, which, according to MacNider,<sup>2</sup> produces a primary lesion of the epithelial cells of the proximal convoluted tubules.

As early as the second day following the injection of the streptococcal nephrotoxin, the dogs passed urine containing albumin, bile and blood (see table 3). At this time there was usually a marked reduction in the elimination of phenolsulphonphthalein, and this depression of renal function was the result of injury to the glomerular structures and not of injury to the tubular, as was revealed by microscopic study of the kidneys of some of the animals.

The functional changes gradually diminished in severity after from three to four days in dogs that received only one injection of nephrotoxin, and the elimination of phenolsulphonphthalein increased. These expressions of functional improvement occurred *pari passu* with the

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2. MacNider, W. de B.: *J. Exper. Med.* 49:387, 1929.

clearing of the glomerular injury. A second and third injection of the nephrotoxin was often followed by urinary abnormalities more severe than those following a single injection (see table 2). However, albumin, blood and casts in marked quantities occurred following the single injection of nephrotoxin. Furthermore, these substances appeared in the urine within a few hours in dogs that had had more than one injection of the nephrotoxin, which might be attributed to a previously induced allergic renal state. Again in these dogs the normal renal function was reestablished much more slowly or the pathologic condition became progressively worse until death. In these animals there was more histologic evidence of glomerular destruction and beginning evidence of retrograde changes in the epithelium of the convoluted parts of tubules. The study of the urinary secretions from the kidneys of these animals permits the deduction that regeneration of tubular epithelium does not occur when the corresponding glomerulus has been destroyed. This may possibly be explained on the ground that the supply of blood to the tubule, which is largely through the efferent branch of the glomerulus, has become cut off by the process in the latter.

#### COMMENT

The results of the experiments here reported are of special interest, since the renal changes were produced with a nephrotoxin that commonly causes nephritis in man. It is also noteworthy that there is a rather complete correspondence between the experimentally induced renal changes and the nephritis of scarlet fever. In consequence a closer analogy can be drawn to the human disease than is possible when improbable excitants of human nephritis, such as uranium nitrate, have been employed. Furthermore, the scarlatinal nephritis in these dogs afforded the opportunity to determine the relative dependence of the urinary abnormalities on certain of the renal changes. While many investigators have produced experimental nephritis in a variety of animals and with a variety of substances, including pathogenic microorganisms and their products, few, it would seem, have attempted to determine the true relationship of the urinary abnormalities to the structural changes in the kidney when common nephrotoxic substances to which man is subject are employed. In this connection the work by Christian,<sup>3</sup> by Pearce and Eisenbrey<sup>4</sup> and by Underhill, Wells and Goldschmidt,<sup>5</sup> among others, would have been of greater clinical value

3. Christian, H. A.: *Tr. Congress Am. Physicians & Surgeons* **9**:1, 1913.

4. Pearce, R. M., and Eisenbrey, A. S.: *J. Exper. Med.* **14**:306, 1911.

5. Underhill, F. P.; Wells, H. G., and Goldschmidt, S.: *J. Exper. Med.* **18**: 347, 1913.

had they employed nephrotoxic agents that commonly cause nephritis in man. As MacCallum<sup>6</sup> pointed out, such substances as uranium nitrate, tartrates, snake venoms and the like are improbable excitants of human nephritis, and therefore the lesions experimentally produced by these may not be at all comparable with those of human nephritis.

In discussing the manner of production of experimental nephritis, the question of allergy comes up for consideration, because it is claimed that allergic lesions in the kidney are inducible with streptococcal antigens. In this connection it is noteworthy that Birkhaug<sup>7</sup> found allergy in a high percentage of rheumatic fever infections obtained with filtrates, autolysates and suspensions of various nonhemolytic streptococci, from which he concluded that there is a common allergenic factor in streptococcal products. Longcope<sup>8</sup> produced specific lesions in the kidney by occasioning in the previously sensitized experimental animal repeated intoxication with protein. Long and Finner<sup>9</sup> succeeded regularly in producing glomerulonephritis by injection of tuberculin into the sensitized experimental animal. Heplar and Simonds<sup>10</sup> reported the results of their work on the experimental production of allergic inflammation in the kidney. In all of the reported instances of experimentally induced allergic nephritis it would seem that the specific lesion has occurred only after repeated injections of the antigenic substance or in animals in which the hypersensitive state existed. Longcope,<sup>11</sup> however, claimed to have produced with a single large dose of foreign protein specific injury to parenchymatous organs, which he ascribed to the gradual splitting of the protein within the animal's body, resulting in the liberation of toxic substances. In this instance, it must be assumed that the allergic reaction was the result of a sensitization of tissue induced by the toxic product first split from the protein. That an allergic reaction accompanied by structural changes may occur simultaneously in the kidney and other organs as well as in the skin is likely in any case in which there exists a specific hypersensitiveness to protein, as has been shown by Gay and Southard,<sup>12</sup> Boughton,<sup>13</sup> Longcope<sup>14</sup> and others.

Bacterial allergy most likely influences the clinical picture in all streptococcal nephritis of man, and particularly the postscarlatinal form.

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6. MacCallum, W. G.: *A Text Book of Pathology*, ed. 4, Philadelphia, W. B. Saunders Company, 1928, p. 262.

7. Birkhaug, K. E.: *J. Infect. Dis.* **40**:549, 1927.

8. Longcope, W. T.: *J. Exper. Med.* **18**:678, 1913.

9. Long, E. R., and Finner, L. L.: *Am. J. Path.* **4**:571, 1928.

10. Heplar, O. E., and Simonds, J. P.: *Am. J. Path.* **5**:473, 1929.

11. Longcope, W. T.: *J. Exper. Med.* **22**:6 and 793, 1915.

12. Gay, F., and Southard, E. E.: *J. M. Research* **16**:143, 1907.

13. Boughton, T. H.: *J. Immunol.* **1**:105, 1916.

14. Longcope, W. T.: *Arch. Int. Med.* **15**:1079, 1915.

In these cases, as shown by Longcope,<sup>15</sup> there are persisting foci of streptococci from which is absorbed a toxic product that on reaching the already sensitized kidneys excites new changes of an allergic nature.

As regards the nephritis reported here I do not believe that allergy could have played any part in the production of the specific renal changes following the first injection of antigen. To explain the nephritis following the primary dose on the basis of an allergic reaction it would be necessary to assume that the animals were already hypersensitive to streptococcal protein. It is not likely that in all of these dogs an allergic state existed because of a previous streptococcal infection. Since the recurrence of acute nephritis in these dogs followed repeated injections of the product of *S. scarlatinae*, these secondary renal reactions could be regarded as allergic, which would support Longcope's conception of the production of postscarlatinal nephritis in man.

Constant and significant structural changes occurred in the dogs in which nephritis was experimentally produced with scarlatinal streptococci or with the toxic product of these organisms. The lesions were glomerular, tubular and interstitial. As a rule, the glomeruli were primarily affected when the toxic principle was in the form of killed culture or "lysate," regardless of whether the injection was made intravenously or intraperitoneally, while, as previously reported,<sup>16</sup> an interstitial type of lesion occurred in animals which had been inoculated with the living streptococci, and in which a generalized infection had developed. The interstitial lesion was an infiltration of the intratubular tissues with lymphocytic cells. Commonly the neutrophils were absent or only few; however, in some of the more advanced stromal lesions they occurred, but were not as numerous as the lymphocytic cells. Associated with the interstitial lesions were viable streptococci, which were readily demonstrable in stained sections and recoverable in pure culture from the fresh tissues. The absence of fibroblasts or of any other evidence of stromal activity in the early interstitial lesion is significant since it indicates that the lymphocytic infiltration was a true reaction of the host to the injurious agent and therefore not reparatory. From this it may be inferred that acute interstitial scarlatinal nephritis of man is the same kind of reaction.

On the other hand, the killed culture or its product produced primarily lesions of the glomerular capillaries, in consequence of which the vascular loops became occluded with thrombi and later adherent to Bowman's capsule. Other glomerular tufts became enlarged through the appearance of numbers of endothelial cells in the lumina of the

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15. Longcope, W. T.: J. Clin. Investigation 5:7, 1927.

16. Duval, C. W., and Hibbard, R. J.: Proc. Soc. Exper. Biol. & Med. 24: 876, 1927.

capillaries. The subcapsular spaces generally contained blood in the form of hyaline masses, also albuminous material and desquamated epithelium. Later, in those subcapsular spaces where hemorrhage had occurred, early proliferation (formation of "crescents") was noted. All these structural changes caused the glomeruli to undergo further and more serious alteration through replacement of the destroyed capillary loops by fibrous tissue.

While alterations in the tubular epithelium were not an early feature in either the glomerular or the interstitial type of experimental scarlatinal nephritis, the tubular epithelium was affected later in the process, degeneration appearing, especially in the epithelium of the convoluted portion of the tubule. Here the lining cells became swollen through the presence of fluid, granules, fat and hyaline droplets. Often the lumen was filled with blood, desquamated epithelium and granular and hyaline casts.

It seems likely that injuries to the glomeruli occasioned by the streptococcal toxic product account for most, if not all, of the urinary abnormalities, since albumin, bile, blood and the substances comprised in casts commonly appear in the urine simultaneously with structural changes that are confined to the renal tufts. The elimination of dye revealed the fact that the chlorides are normally filtered through the glomeruli and that the tubular epithelium has little or no excretory power for this salt. There was considerable evidence to show that the normal tubular epithelium is concerned mainly with the altering of the filtrate from the glomeruli through selective absorption by the lining cells. It is further to be remarked that the secretion of uric acid was not affected in these animals, though in all of them there was marked glomerulonephritis without structural changes in the tubules. Thus it would seem that the tubular epithelium, and not the tufts, have to do with the concentration of glomerular filtrate, selecting out and turning back into the circulation water, chlorides, etc., since they always appeared histologically normal when the glomeruli were badly injured.

Dogs with acute glomerulonephritis and no tubular changes excreted little urine as compared with the normal amount, indicating that the function of the tubular epithelium was unimpaired. Albumin and blood, undoubtedly have their origin in the glomerular tufts, as these substances occurred only when the capillaries were injured. Combined glomerular and tubular lesions did not quantitatively affect these abnormal substances in the urine. Albuminuria and hematuria always signified well defined alterations in the capillaries of the tufts, which was confirmed by histologic study. Casts were numerous and early in the streptococcal glomerulonephritis, because of the injured capillary tufts from which presumably these substances are derived, and by reason



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of the fact that their formation is aided in the lumina of the tubules through the abstraction of water by the normal tubular epithelium.

Dogs in which there was induced an acute glomerular nephritis with the toxic product of scarlatinal streptococci returned, after a period of weeks, to a normal function, even though microscopic study of the sections of the kidney, still showed persistence of glomerular and tubular injuries, both acute and chronic. The ability of such animals to regain the normal function under these circumstances depended apparently not on regeneration, but on the fact that much of the renal parenchyma had escaped injury and was adequate for the carrying on of the renal function that is consistent with health. Dogs that were unable to effect a return to normal function after they had shown all the clinical evidence of acute glomerular nephritis and had finally succumbed may be regarded as suffering from a severe primary effect which involved too great a number of the glomeruli. These dogs showed the functional expression and structural changes incident to chronic diffuse nephritis.

#### CONCLUSIONS

In all essentials the renal lesions of experimental scarlatinal nephritis in the dog correspond to those of scarlatinal nephritis in man. In dogs the toxic product of *S. scarlatinae* has a selective action on the kidney resulting commonly in acute glomerulonephritis. This nephrotoxin primarily affects the endothelium of the glomerular blood vessels, causing in them an albuminous and fatty degeneration. The capillary endothelial lesions are frequently followed by hyaline thrombi and the rupture of the blood vessels with hemorrhage into the subcapsular space. Secondary changes of a retrograde character occur in the tubular epithelium, more especially in that of the convoluted portion.

The "killed" culture of scarlatinal streptococci acts in the same manner as the lysate in the production of experimental nephritis. A single large dose of this nephrotoxin often causes a fatal acute hemorrhagic glomerulonephritis. Furthermore, in dogs surviving the acute nephritis from a single dose chronic diffuse nephritis commonly develops weeks and months afterward. "Living" scarlatinal streptococci cause, in addition to glomerular lesions, an interstitial nephritis characterized by an intertubular lymphocytic infiltration.

The renal injury produced by one dose of scarlatinal nephrotoxin is always aggravated by a second injection. Such an exacerbation may be regarded as allergic, since the primary dose may have induced a hypersensitive state of the kidney. However, it is my belief that the lesions following the first dose are not allergic, but are caused by the destructive action of the streptococcal poison.

The urinary findings in acute nephritis in the dog are in general a fairly reliable index to the character and location of the renal lesions. Blood in the urine indicates hemorrhage into the subcapsular space. Hyaline casts are indicative of an impairment of glomerular function, which increases as the injury becomes intensified. Granular casts signify degeneration of the tubular epithelium. Albumin appears in the urine when there are degenerative changes of the glomeruli. Severe injury to the glomerular structures invariably causes a reduction in the amount of urine, while additional injury to the tubular epithelium does not further depress elimination.

The experimental nephritis produced in the dog with the toxic product of *S. scarlatinae* has afforded the opportunity to trace the progress and relationship of the various anatomic changes, and to correlate these with renal physiologic disturbances as expressed by abnormal substances in the urine.

# ANOMALOUS ORIGIN AND COURSE OF THE LEFT CORONARY ARTERY \*

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Anomalies of the origin and course of the coronary arteries are common, but the particular anomaly described here is so rare that reporting it seems justifiable.

A man, aged 65, came to the Mayo Clinic because of symptoms of urinary obstruction due to benign hypertrophy of the prostate gland. Prostatectomy was performed, and six days afterward the patient died. Death was due to embolic pneumonia and renal insufficiency.

Examination of the heart at necropsy disclosed only one coronary orifice. This was situated behind the right anterior aortic cusp, in the position normally occupied by the orifice of the right coronary artery. It was considerably larger than the normal coronary orifice, measuring 4.5 mm. in diameter, as against a normal orifice of 3 mm. This was found to be the common orifice for the two arteries, the larger of which curved toward the right, to enter and follow the right part of the coronary sulcus to the posterior surface of the heart. All branches of sufficient size to permit being opened or probed were traced carefully; the course and distribution appeared to be identical with that of the normal right coronary artery.

The smaller artery, which must be regarded as an anomalous left coronary artery, passed downward and to the left, at the root of the aorta and beneath the muscle of the posterior wall of the right ventricle. In this situation it was about 1 cm. below the valves of the pulmonary artery. It passed directly to the left, lost its relationship to the anterior wall of the aorta and took its course through the muscle of the right ventricle. On reaching the interventricular septum, it curved anteriorly in the right half of the septum, to reach the anterior surface of the heart. Three millimeters deep in the septum it gave off a branch, which passed downward through the muscle for a distance of 1 cm.; there it became superficial and continued its downward course to the apex.

Immediately after the main branch emerged from the interventricular septum, it divided into two branches, an ascending branch and a descending branch. The ascending branch, which was the larger, turned acutely upward and slightly to the right for a short distance. Continuing its upward course, it then curved slightly to the left to reach the left part of the coronary sulcus. From this point

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\* From the Section on Pathologic Anatomy, the Mayo Clinic.

it followed the course of the normal circumflex branch of the left coronary artery to the posterior surface of the left ventricle. The descending branch took its course downward and to the left, and gave rise to several smaller vessels. These small vessels definitely descended toward the apex. The two descending branches of the left main artery appeared to furnish the blood supply to most of the area which is normally supplied by the anterior descending branch of the left coronary artery (figs. 1 and 2).

Abbott,<sup>1</sup> in her extensive consideration of congenital cardiac disease, stated that both coronary arteries may arise behind a single aortic cusp, or that one coronary artery may be absent. However, she did not give definite details. Bochdalek<sup>2</sup> described a case in which there was one coronary orifice behind the right anterior cusp of the aorta, and from this there arose one abnormally large artery, which, after a short distance, divided into three branches; the first took the course that is normally taken by the right coronary artery; the second took the course of the circumflex branch of the normal left coronary artery, and the third passed to the left, through the muscle of the posterior wall of the right ventricle. It finally passed anteriorly, through the interventricular septum, to reach the surface of the heart, and turned downward, taking a course similar to that of the anterior descending branch of the left coronary artery.

Engelman<sup>3</sup> quoted Hyrtl, Thebesius, Otto and Cruveilhier, who had observed that the two coronary arteries may arise either from a common trunk or from one sinus of Valsalva. He recorded a case of his own in which there was only one coronary orifice; it was situated behind the left anterior aortic cusp, and from this, one large artery arose which soon divided into two branches. One branch passed to the left, as does the circumflex branch of the normal left coronary artery. The other branch passed downward for a distance of 0.5 cm., where a relatively small branch passed to the right between the roots of the aorta and pulmonary artery and continued its course in the right coronary sulcus to the posterior surface of the heart. Engelman considered this branch as the right coronary artery arising from a branch of the

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1. Abbott, Maude E.: *Congenital Cardiac Disease*, in Osler, William; and McCrae, Thomas: *Modern Medicine*, ed. 3, Philadelphia, Lea & Febiger, 1927, vol. 4, p. 794.

2. Bochdalek: *Anomaler Verlauf der Kranzarterien des Herzens*, Virchows Arch. f. path. Anat. **41**:260, 1867.

3. Engelman, Guido: *Ein Fall von mangelhafter Coronararterie*, Anat. Anz. **14**:348, 1897-1898.

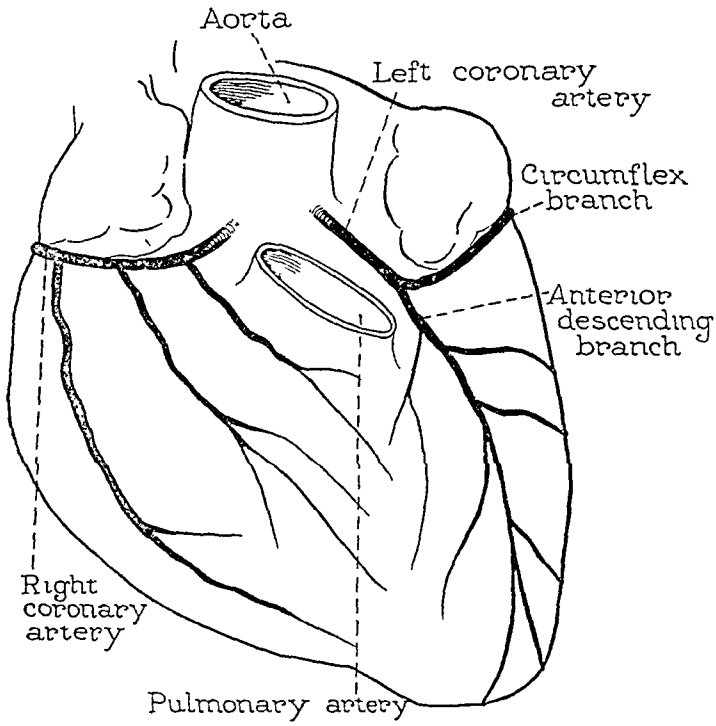


Fig. 1.—Anterior view: normal origins and courses of the coronary arteries.

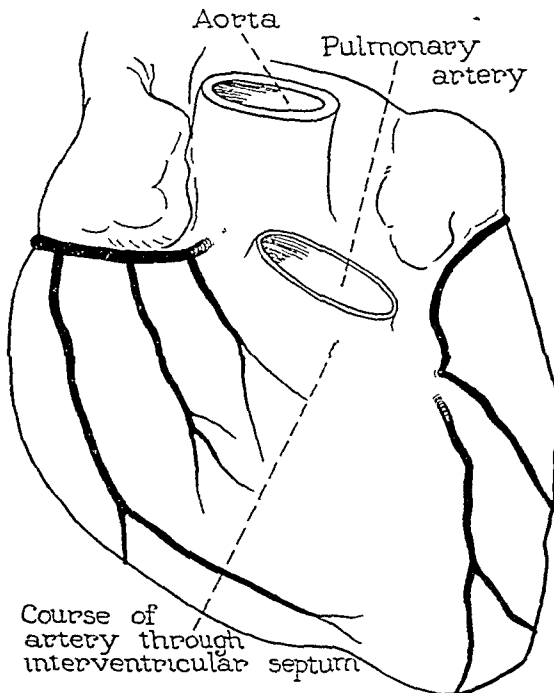


Fig. 2—Origin and courses of coronary arteries described.

left coronary artery. The main descending branch continued its course downward for a distance of 0.5 cm., where it divided into two branches. The smaller of these branches took the course of the anterior descending branch of the normal left coronary artery. The larger branch, which he called an abnormal branch, passed downward and slightly to the right, over the anterior surface of the right ventricle, and continued over the right border of the heart to the posterior surface of the right ventricle.

Gallavardin and Ravault <sup>4</sup> reported a case apparently identical with the one that I have described.

Smith and Graber <sup>5</sup> reported the congenital absence of the left coronary artery. A small artery, coursing to the left, was given off by the main artery.

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4. Gallavardin, L., and Ravault, P.: *Anomalie d'origine de la coronaire antérieure*, Lyon méd. **136**:270, 1925.

5. Smith, F. M., and Graber, V. C.: *Coronary Thrombosis with Congenital Absence of the Left Coronary Artery*, Arch. Int. Med. **38**:222, 1926.



# HOMOIOTRANSPLANTATION AND HETEROTRANS- PLANTATION IN THE GUINEA-PIG

EFFECTS OF GRADED DEGREES OF HEAT ON CARTILAGE AND ON  
THYROID GLAND \*

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In a series of papers Loeb has analyzed the factors that determine the reaction of the host against transplanted tissue.<sup>1</sup> These observations established the fact that there are characteristic qualitative and quantitative differences in the reaction of the host against homoiotransplanted, as compared with that against heterotransplanted, tissues. Loeb attributed these characteristic reactions of the host to substances given off by the transplanted tissues, and he designates these substances as homoiotoxins and heterotoxins, which differ from each other in the intensity and also in the character of the reactions which they call forth. There were strong indications that typical homioireactions were elicited only by living tissue, whereas heteroreactions were called forth also by nonmetabolizing tissue, such as coagulated blood. It is of interest in this connection that Loeb and Drake found it possible to produce various grades of injury in amebocyte tissues by heating the tissues within a relatively small range of temperature and time of exposure, and by this means were able to reduce the activity of the cells to various stages intermediate between normal function and death.<sup>2</sup> Similarly in his earlier experiments, Loeb was able to reduce the growth energy of transplantable tumor tissue by subjecting the tissue to graded degrees of heat and also to the action of certain chemicals before transplantation.<sup>3</sup>

It was thought, then, to be of importance to determine if by reducing the activity of various normal tissues by exposing them to graded intensities of heat before homoiotransplantation, the reaction of the host would be decreased in proportion and whether any parallelism could be found between the reduction in growth activity of the transplant and the diminution in the intensity of the reaction of the host. In

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\* From the Department of Pathology, Washington University School of Medicine.

1. Loeb, Leo: Transplantation and Individuality, *Physiol. Rev.* **10**:547, 1930.

2. Loeb, Leo; and Drake, Dorothy: *J. M. Research* **44**:447, 1924.

3. Loeb, Leo: *J. M. Research* **8**:44, 1902; *Virchows Arch. f. path. Anat.* **172**: 345, 1903; *Am. Med.* **5**:412, 1903; **10**:265, 1905. Corson-White, E. P., and Loeb, Leo.: *Centralbl. f. Bakt.* **56**:488 and 325, 1910.

addition it was hoped that an answer would be found to the question whether any differences exist in these regards between the behavior of tissues that have a great potentiality to proliferate mitotically, such as those of the thyroid gland, and that of tissues that have less capacity to grow by mitotic division, such as cartilage; and, also it was hoped to study any differences that may exist in these respects between homoiotransplantation and heterotransplantation.

#### METHOD

For this purpose the thyroid glands and pieces of xiphoid cartilage of guinea-pigs and of rats were excised and pieces placed in test tubes containing sterile Ringer's solution. These test tubes were then immersed in a water bath of constant temperature, which had been previously heated to the desired temperature; the various temperatures used were 43, 45, 47, 49 and 51 C. Series of pieces of tissue were allowed to remain in the waterbath at each of these temperatures for fifteen, thirty and forty-five minutes, respectively. The pieces were then transplanted into subcutaneous pockets of guinea-pigs. In the case of heterotransplantation thyroid glands and cartilage of rats were transplanted into guinea-pigs. In every case the transplants were removed after twenty days, and sections were made for microscopic study.

#### HOMOIOTRANSPLANTATION OF THYROID GLAND

*Heated at 43 C.*—The recovered thyroid gland homoiotransplants that had been heated at 43 C. for fifteen, thirty and forty-five minutes, respectively, consisted in each case, chiefly of a central necrotic portion in which some colloid masses were seen, a few of which were lined by living acinar cells. This central necrotic portion was surrounded by fibrous tissue in which no remaining thyroid gland tissue was found. There were a number of clumps of lymphocytes and many scattered lymphocytes throughout the connective tissue. The surrounding transplanted fat tissue was fairly well preserved, but some scattered as well as massed lymphocytes and a small amount of connective tissue had invaded it. There were no essential differences between the transplants that had been heated for fifteen, thirty and forty-five minutes, respectively.

*Heated at 45 C.*—The homoiotransplants that had been heated at 45 C. for fifteen, thirty and forty-five minutes, respectively, consisted, in each case, chiefly of a small necrotic central portion, surrounded by a great mass of dense fibrous tissue through which there was scattered only a rather small number of lymphocytes. Connective tissue had penetrated into and replaced the greater part of the necrotic central portion of the transplanted tissue. There were a few remnants of thyroid gland acini, among which a few epithelial cells were still well preserved. However, no colloid was seen in any of the sections. The surrounding fat tissue was fairly well preserved, but many foreign body

giant cells were seen, replacing portions of fat tissue. No essential differences existed between the various specimens exposed for the different periods of time.

On the whole in these specimens, as compared with those that had been heated at 43 C., there was a rather definite decrease in the number of lymphocytes, with an increase in the amount of connective tissue. Giant cells were also more abundant in the fat tissue in this series than in the former one.

*Heated at 47 C.*—In the homoiotransplants that had been heated at 47 C. for fifteen, thirty and forty-five minutes, respectively, some differences in appearance were noted that accorded with the time of heating. In those that had been heated for fifteen minutes there was found chiefly a completely fibrosed mass of tissue, through which were scattered considerable numbers of single lymphocytes, as well as small masses of lymphocytes. The central necrotic mass, as well as the surrounding fat tissue, was almost entirely replaced by fibrous tissue. No thyroid gland tissue or colloid remained.

The transplants that had been heated for thirty minutes consisted chiefly of loose vascular connective tissue surrounding a central dense fibrous mass. Lymphocytes were scarce in these sections. The transplanted fat tissue was well preserved, and there was practically no reaction on the part of the connective tissue and lymphocytes, nor were there giant cells about it. No thyroid gland tissue remained.

The transplants that had been heated for forty-five minutes showed no trace of thyroid gland; lymphocytes were practically absent. However, there was a small amount of connective tissue present, and the transplant consisted chiefly of fat and rather loose connective tissue. The fat was being phagocytosed by large mononuclear cells.

*Heated at 49 C.*—The homoiotransplants that had been heated at 49 C. for fifteen minutes consisted chiefly of necrotic material and of fat tissue, which was being phagocytosed by large mononuclear cells, or of fibrous tissue that had replaced the necrotic tissue. There was only a slight connective tissue reaction. No thyroid gland tissue remained preserved. Lymphocytes were practically absent in the tissue. While those heated for fifteen minutes showed considerable necrosis and only a small amount of connective tissue, those heated for forty-five minutes showed no necrotic tissue and a large amount of connective tissue. In this case the connective tissue appeared to have entirely replaced the central necrotic mass that was noticeable in practically the whole former series.

*Heated at 51 C.*—The pieces of thyroid gland that had been heated at 51 C. for fifteen, thirty and forty-five minutes and then transplanted showed no essential difference from those that had been heated at 49 C.

Therefore, on the whole in this series, the lymphocytic reaction of the host in homoiotransplantation of thyroid gland varied inversely with the increased degree of heating, while the connective tissue reaction varied directly with the increased degree of heating; the greater the degree of heating the greater was the connective tissue reaction. However, since no thyroid gland remained preserved in the specimens heated to the higher degrees, it is not possible to ascertain whether the reaction noted occurred at a time when some tissue of the thyroid gland remained or whether it occurred subsequently to the destruction of the thyroid gland tissue. Additional experiments in which the time of examination is varied are necessary for the determination of this point.

#### HETEROTRANSPLANTATION OF THYROID GLAND

*Heated at 43 C.*—In the rat thyroid glands that had been heated at 43 C. for fifteen, thirty and forty-five minutes, respectively, and then transplanted to guinea-pigs there was found chiefly a very vascular connective tissue in which no remnants of preserved thyroid gland tissue were seen. Lymphocytes and polymorphonuclear leukocytes were abundant in all the sections, but the relative proportions of the lymphocytes and the polymorphonuclear leukocytes varied; in some specimens polymorphonuclear leukocytes predominated, while in others the lymphocytes were more numerous. The fat tissue was to a large extent replaced by connective tissue. Only occasional giant cells were seen.

*Heated at 45 C.*—The heterotransplants that had been heated to 45 C. for fifteen, thirty and forty-five minutes were found to be very similar to those described in the foregoing paragraph, except that the lymphocytes were perhaps somewhat less numerous in the series heated at 45 C., so that the polymorphonuclear leukocytes predominated in the majority of the sections.

*Heated at 47 C.*—In the heterotransplants heated at 47 C. for fifteen, thirty and forty-five minutes again no preserved thyroid gland tissue was found. They consisted in each case chiefly of a mass of vascular connective tissue in the center of which there was a small area of necrosis which contained varying numbers of polymorphonuclear leukocytes and necrotic débris. The lymphocytes were somewhat fewer and more scattered as compared with those in the transplants that had been heated at 45 C. The polymorphonuclear leukocytes were also somewhat decreased in number, but the connective tissue reaction was, on the whole, somewhat greater.

*Heated at 49 and 51 C.*—The heterotransplants that had been heated at 49 and 51 C. for fifteen, thirty and forty-five minutes consisted chiefly, in each case, of a mass of vascular connective tissue among the cells of which there were considerable numbers of scattered lymphocytes

and polymorphonuclear leukocytes. The fat tissue was invaded by large mononuclear phagocytic cells, fibroblasts and some polymorphonuclear leukocytes. Giant cells were scarce. No remnants of thyroid gland were seen.

On the whole, I found very noticeable differences between the reactions of the host to heterotransplants as compared with those to homoiotransplants, so far as in the former specimens polymorphonuclear leukocytes were found in great numbers, while in the latter specimens they were scarce or entirely absent. Furthermore, the heating of the heterotransplants was effective to only a slight degree in reducing the polymorphonuclear and lymphocytic reactions of the host, while the lymphocytic reaction of the host to homoiotransplants was reduced very markedly by the heating. In heterotransplantation as in homoiotransplantation the connective tissue reaction was greater in the specimens that had been heated at the higher degrees of temperature, as compared with those that had been heated at the lesser degrees.

#### HOMOIOTRANSPLANTATION OF CARTILAGE

*Heated at 43 C.*—The homoiotransplanted cartilage that had been heated at 43 C. for fifteen, thirty and forty-five minutes consisted of cartilage, perichondrium and the surrounding fat and connective tissue, all of which were in a fair state of preservation. Surrounding the transplant there was a marked lymphocytic and connective tissue reaction, which extended into the fat tissue and slightly into the cartilage, especially in areas in which some injury had occurred in the latter. There were also noted occasional giant cells in the surrounding fat tissue. On the whole, these transplants appeared similar to homoiotransplants of unheated cartilage removed at the same time.

*Heated at 45 C.*—In the homoiotransplanted cartilage and surrounding tissue that had been heated at 45 C. for fifteen minutes, the results were similar to those described in the foregoing paragraph: no effects of the heating were observable. On the other hand, the cartilage transplants that had been heated for thirty minutes showed what was interpreted as mild effects of the heating. The cartilage cells were no longer clearly discernible in the red-staining peripheral areas, although in the large majority of cases in the more central areas the transplanted cartilage cells were still fairly well preserved. There was a marked lymphocytic and connective tissue reaction, which in some cases extended into the cartilage.

The transplanted cartilage that had been heated for forty-five minutes was also in a fair state of preservation, although the zone of destroyed cartilage was somewhat greater here than in the cartilage that had been heated for only thirty minutes. As far as the number of lymphocytes

or the intensity of the connective tissue reaction was concerned there was no appreciable difference between these specimens and the previously described grafts.

*Heated at 47 C.*—Heating for fifteen minutes at 47 C. resulted in still greater injury to the cartilage cells, the cartilage now staining deeply with eosin throughout, instead of with hematoxylin in the central parts, as is usually the case in the areas where the cartilage is thicker. Cartilage cells were scarce, although the perichondrial cells appeared little altered. Lymphocytes were present, but in numbers definitely less than in those transplants that had been heated at 45 C. The connective tissue reaction showed no appreciable quantitative change. The transplanted connective tissue surrounding the cartilage was hyalinized and was in the process of replacement by newly formed connective tissue of the host. Those specimens that had been heated for thirty minutes generally showed the cartilage cells to be almost entirely destroyed, whereas the perichondrial cells remained well preserved and in the majority of cases displayed some proliferative activity. Hypertrophic spindle-shaped cells penetrated into the destroyed cartilage and formed new cartilage. In order to determine whether this regeneration of cartilage occurred specifically in those transplants that had been heated at 47 C. for thirty minutes, an additional series of experiments was carried out in which the cartilage was heated at 47 C. for fifteen, thirty and thirty-five minutes. Six pieces were heated for thirty-five minutes, six for fifteen minutes and twelve for thirty minutes. Seven of the twelve that had been heated for thirty minutes showed some regeneration of cartilage, whereas three of the six that had been heated for fifteen minutes showed it, and only one of the six that had been heated for thirty-five minutes showed it.

The lymphocytic reaction throughout this series was either entirely absent or present only as a few scattered cells in the surrounding fat and connective tissue. The connective tissue reaction was also diminished to some extent, although the greater part of the fat and connective tissue that surrounded the cartilage had been replaced by loose vascular connective tissue of the host.

It seems that in heating the cartilage for from fifteen minutes to thirty-five minutes at 47 C. before homoiotransplantation the cartilage was injured to such a degree that it became necrotic following transplantation, whereas the perichondrium evidently remained alive. Then, in accordance with the observations of Loeb,<sup>4</sup> the contact with the necrotic cartilage stimulated the perichondrial cells to proliferate to form new cartilage cells, which in some cases pushed their way into and replaced considerable amounts of the necrotic cartilage. It seems that this

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4. Loeb, Leo: *Am. J. Path.* 2:111 and 315, 1926.

regeneration of cartilage occurred at its optimum in the specimens that had been heated at 47 C. for thirty minutes.

In those heated at 47 C. for forty-five minutes, the entire transplanted tissue, including cartilage matrix, cartilage cells, perichondrium and the surrounding fat and connective tissue stained homogeneously with eosin. There were no preserved cells in the transplanted tissue. There was practically no connective tissue or lymphocytic reaction noticeable, and the transplant lay as an inert mass in the meshes of a network of loose edematous connective tissue.

*Heated at 49 and at 51 C.*—The homoiotransplants heated at 49 and at 51 C. for fifteen, thirty and forty-five minutes showed essentially the same picture as those that had been heated at 47 C. for forty-five minutes. Occasionally a few lymphocytes were seen, and occasionally the connective tissue was somewhat dense, instead of loose and edematous. Phagocytic cells and foreign body giant cells penetrated in rather large numbers into the peripheral portions of the transplanted tissue.

#### HETEROTRANSPLANTATION OF CARTILAGE

*Heated at 43 C.*—The heterotransplants of cartilage that had been heated at 43 C. for fifteen, thirty and forty-five minutes consisted in each case of a necrotic mass of cartilage surrounded by masses of lymphocytes and polymorphonuclear leukocytes and connective tissue. No cartilage cells remained. The cells of the perichondrium in a few cases, however, appeared fairly well preserved. There was no regeneration of cartilage.

*Heated at 45 C.*—The heterotransplants that had been heated at 45 C. for fifteen, thirty and forty-five minutes showed no preserved cartilage cells; the cells of the perichondrium were also destroyed. There were considerable numbers of scattered lymphocytes, and a few clumps of these cells and some polymorphonuclear leukocytes were present. Both of these elements were perhaps somewhat decreased as compared with the transplants that had been heated at 43 C. On the other hand, the connective tissue reaction was possibly somewhat greater here than in the former series.

*Heated at 47 C.*—The heterotransplants that had been heated at 47 C. for fifteen, thirty and forty-five minutes presented a picture very similar to that seen in the heterotransplants that had been heated at 45 C. The cartilage and the surrounding fat and connective tissue were entirely necrotic, and there were considerable numbers of lymphocytes and occasional polymorphonuclear leukocytes in the surrounding tissues. There was little penetration of these cells into the cartilage. The connective tissue reaction appeared to be slightly stronger in these than in the transplants heated at 43 and at 45 C.

*Heated at 49 and at 51 C.*—Pieces of cartilage heated at 49 and at 51 C. for fifteen, thirty and forty-five minutes appeared very similar to those heated at 47 C. Each consisted of necrotic cartilage which lay in the center of a network of edematous hyaline fibrous tissue through which were scattered considerable numbers of lymphocytes and polymorphonuclear leukocytes. Occasional giant cells were seen in the tissues that surrounded the transplant.

#### CONCLUSIONS

Heating thyroid gland at degrees of heat ranging from 43 to 51 C. preceding homoiotransplantation causes a marked lessening of the lymphocytic reaction of the host ordinarily occurring after homoiotransplantation. The connective tissue reaction, however, appears to be somewhat increased with the heating to the higher degrees.

Heating thyroid gland at graded degrees preceding heterotransplantation is only slightly effective in diminishing the reaction of polymorphonuclear leukocytes and lymphocytes of the host ordinarily occurring after heterotransplantation. The connective tissue reaction also appears to be somewhat greater here than that ordinarily occurring after heterotransplantation of unheated thyroid gland.

Exposing cartilage to graded degrees of heat preceding homoiotransplantation causes a marked and graded decrease in the lymphocytic reaction as well as in the connective tissue reaction of the host ordinarily occurring after homoiotransplantation of cartilage. This reduction in the reaction of the host is concomitant with a marked increase in the growth activity of the transplanted cartilage, as manifested by hypertrophy and hyperplasia of the perichondrial cells, which proliferate and lay down new cartilaginous matrix. This regeneration of cartilage occurs at its optimum in transplants of cartilage that have been heated at 47 C. for thirty minutes before transplantation.

Heating cartilage at graded degrees of heat preceding heterotransplantation is only slightly effective in causing a reduction in the polymorphonuclear leukocytic and lymphocytic reactions of the host ordinarily occurring after heterotransplantation. The connective tissue reaction on the part of the host against the transplanted tissue appears to be somewhat increased in amount over that which occurs ordinarily against unheated cartilage.

Homoiotransplants killed before transplantation by mild degrees of heat no longer elicit a homioireaction, while heterotransplanted tissue, under the same conditions, still calls forth a definite heteroreaction on the part of the host.



# Laboratory Methods and Technical Notes

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## METHOD FOR EXAMINING THE APPENDIX

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### DEMONSTRATION OF A PERFORATION OF THE APPENDIX

Perforations in gangrenous appendixes are not as easily demonstrable as is believed. In my experience, gangrenous appendixes that presumably should be perforated were not, and vice versa. Even the histologic picture is frequently deceptive. What appears to be a frank necrosis



A method of demonstrating the presence and location of a perforation in an appendix.

involving all the coats of a part of an appendix fails to reveal a perforation. The method suggested allows the determination of a perforation and the localization of the lesion.

An ordinary 5 or 10 cc. syringe is partly filled with a weak solution of eosin. A needle is attached to the syringe, and the needle is introduced into the lumen of the appendix through its proximal end. A hemostat is applied over the appendix and needle to keep the needle in place and to prevent the escape of the eosin (fig.). The hemostat is applied over that part of the appendix which shows the hemostat markings made by the surgeon. The piston of the syringe is gently pushed down so that the eosin solution runs into the appendical lumen. At the point of perfora-

\* Submitted for publication, June 29, 1931.

\* From the Laboratories and Department of Medical Research, Toledo Hospital.

tion, the eosin escapes through the wall and marks the point of the perforation. This method of filling the appendix was found preferable to the introduction of the fluid by gravity. The slight pressure exerted was not found to produce artificial perforations in gangrenous appendixes. If a permanent record is desired of the perforation and its location, iodized poppy seed oil 40 per cent may be introduced instead of eosin and a roentgenogram taken.

#### A METHOD OF CUTTING AND SECTIONING THE APPENDIX

The method that is commonly employed in cutting appendixes for gross and histologic study consists in making several transverse sections across the length of the organ. Such a method allows only an inspection of isolated parts and destroys relationships. If ten sections are made, the chance of locating a lesion histologically is 1:100,000 in an appendix 6 cm. in length and with sections 6 microns in width. The following method permits gross inspection of the lumen and of the wall of the entire organ and preserves the relationship of lesions to normal areas.

After the appendix is received from the operation room, it is placed in a 10 per cent formaldehyde solution for from six to twenty-four hours. The organ is then removed from the fixative and cut longitudinally with a long and flat-bladed knife. The cut is begun at the tip of the appendix with the heel of the knife and carried longitudinally through the approximate middle of the organ. The appendix is supported gently with the left hand, and the knife is carried in a single cut to avoid an irregular surface. At the completion of the section, two equal halves are obtained. Each half of the organ shows the lumen, its contents and the wall.

For histologic sections one or both halves may be used. Any method of handling the tissue preparatory to embedding may be employed. However, in my experience, it is preferable to use alcohol for dehydration and chloroform for clearing. Either celloidin or Warthin's celloidin sheet method or paraffin embedding may be selected. The paraffin method requires more skill and care in preventing wrinkles and tears. Sections 6 microns in width are made through various levels in which the appendical lumen persists. From five to ten sections will give a composite picture of the entire organ. The sections allow the determination of the width of the lumen and the relation of the opposing surfaces, and make it possible to study the whole length of the appendix in a single or two sections.

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#### THE USE OF BUTYL ALCOHOL IN THE PREPARATION OF PARAFFIN SECTIONS\*

WILLIAM A. HEWITT, SAN FRANCISCO

Tissue properly fixed in 10 per cent formaldehyde (any other fixative may be used) is rinsed in tap water to remove excess formaldehyde and then treated by immersion in 50 per cent alcohol (ethyl or methyl) for two hours, then in 80 per cent alcohol (ethyl or methyl) for two hours and then in 95 per cent alcohol (ethyl or methyl) for two hours or over night.

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\* From the Department of Pathology, University of California Medical School.

From 95 per cent alcohol the tissues are put directly into butyl alcohol (azo or normal), two changes covering a period of six hours, or the tissues may be left in the butyl alcohol over night without harmful results. From butyl alcohol the tissues are placed directly in paraffin (melting point from 56 to 58 C.), about three changes covering a period of twenty-four hours. They are then embedded. If one desires to put sections through more rapidly, as is required with surgical specimens, the same procedure can be followed by keeping all solutions in the oven at from 56 to 58 C. Sections can be completed in thirty-six hours.

The advantages of butyl alcohol are as follows: There is little of the shrinkage of tissue that occurs with absolute alcohol and xylene. Tissue can be left in butyl alcohol for from a day to a week, without harmful results. Large sections can be cut and ribboned with ease. The elimination of absolute alcohol and xylene means a tremendous saving in expense, for there is little evaporation of butyl alcohol, and a gallon, costing approximately \$3.50, will do the same amount of work as 4 gallons of xylene and 5 gallons of absolute alcohol, costing approximately \$30.

# General Review

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## THE ETIOLOGY OF POSTVACCINIAL ENCEPHALOMYELITIS \*

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### SYNOPSIS

#### Clinical Picture

#### Epidemiology

- Period of Incubation

- Incidence in Relation to Number of Vaccinations

- Grouping of Cases

- Rural Distribution

- Incidence Considered in Relation to Strain of Virus Used, and in Relation to Reaction at Site of Vaccination

- Incidence in Relation to that of Other Nervous Diseases

- Incidence in Relation to Age

#### Morbid Anatomy

#### Possibly Related Conditions

- Encephalomyelitis Complicating Smallpox

- Encephalomyelitis Complicating Measles

- Nervous Complications of Varicella

- Nervous Complications of Mumps

- Other Postinfection Encephalitis

- Paralysis Complicating Antirabic Treatment

- Independent Conditions

#### Theories Regarding the Etiology of Postvaccinial Encephalitis

- Accidental Relationship

- Vaccinia Virus as the Direct Cause

- Activation of Some Other Virus

- Allergy

#### Prophylaxis and Treatment

#### Summary

The occurrence, in recent years, of a serious acute disease of the central nervous system complicating the oldest and as yet the most effective of the procedures for immunization has attracted widespread concern. Although general attention was first called to the possibility that such a complication occurs by the report of Lucksch in 1924,<sup>1</sup>

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\* Submitted for publication, Feb. 7, 1931.

\* From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

1. Lucksch, Franz: *Med. Klin.* **20**:1170, 1924.

it was apparently first recognized in 1922 in England when four patients with an acute fatal nervous condition and a history of recent vaccination were admitted to the London Hospital. The essential lesions, as found at autopsy, were those of an acute diffuse nonpurulent encephalomyelitis. Further inquiry revealed seven similar cases in the London area. Turnbull and McIntosh, in 1926,<sup>2</sup> reported these cases with full pathologic observations, and included the reexamination of a similar case that occurred in 1912. During the summer of 1923, fifty-one other cases were reported in various districts throughout England, and in November of that year the Andrewes committee was appointed by the Minister of Health to investigate the condition.<sup>3</sup>

In Bohemia, in 1923, Lucksch<sup>4</sup> performed autopsies in three cases of nervous disease closely following vaccination, and at first considered the pathologic changes to be those of epidemic encephalitis. In Holland, in 1924 and 1925, reports of thirty-five cases were collected by Bastiaanse,<sup>5</sup> and sporadic cases were reported from various other countries in Europe (Stiner,<sup>6</sup> Hauswirth,<sup>7</sup> Frommel and Baumgartener,<sup>8</sup> Jehle,<sup>9</sup> Report of League of Nations Commission on Vaccination<sup>10</sup>).

Since these earlier reports, a considerable number of cases have occurred in various parts of the world, and attempts have been made to reconstruct from records cases that occurred before such a possibility was recognized. Holland and England have had by far the greater number of cases; only in these countries and possibly in Germany has the condition approached epidemic proportions. Jitta<sup>11</sup> gave a total of 197 cases reported in Holland to and including 1928, with the peak of the incidence curve lying between 1924 and 1927. A considerable number of cases was still being reported to September, 1929,<sup>12</sup> making a total of well over 150 definite cases to that time. In the combined Andrewes-Rolleston reports<sup>3</sup> on the disease in England, 93 definite cases were reported to and including 1927, most of them occurring in

2. Turnbull, H. M., and McIntosh, James: *Brit. J. Exper. Path.* **7**:181, 1926.

3. Report of Ministry of Health Committee on Vaccination, London, His Majesty's Stationery Office, 1928.

4. Lucksch, Franz: *Centralbl. f. Bakteriol. (Abt. 1)* **96**:309, 1925.

5. Bastiaanse, F. S. van B.: *Bull. Acad. de méd., Paris* **94**:815, 1925.

6. Stiner, O.: *Schweiz. med. Wchnschr.* **6**:244, 1925.

7. Hauswirth, A.: *Schweiz. med. Wchnschr.* **7**:1113, 1926.

8. Frommel, E., and Baumgartener, J.: *Schweiz. med. Wchnschr.* **7**:857, 1926.

9. Jehle, quoted by Lucksch: *Centralbl. f. Bakteriol. (Abt. 1)* **103**:227, 1927.

10. Report of League of Nations Commission on Vaccination, 1928, League Publications, CH, 739.

11. Jitta, N. M. J.: *Bull. de l'Office internat. d'hyg. pub.* **22**:51, 1930.

12. Editorial, *Lancet* **2**:1154, 1929.

1923. Sporadic reports subsequent to that time brought the number to over 100 at the end of 1929. Germany had a total of approximately 40 cases from 1921 to 1930, with the majority in the period from 1927 to 1929.<sup>13</sup> Three cases were reported by Duken<sup>14</sup> and Widowitz<sup>15</sup> as late as June, 1930. Sweden to 1929 had had a total of about 25 cases, with 9 occurring in 1928.<sup>16</sup> Vienna and lower Austria contributed 28 cases from 1925 to 1929, 11 in 1929 (Knöpfelmacher,<sup>17</sup> Zappert<sup>18</sup>). In other countries in which the disease has occurred, there have been chiefly isolated and sporadic cases: France, 9;<sup>19</sup> Poland, 2;<sup>20</sup> Norway, 11;<sup>21</sup> Italy, 26; Japan and Argentina;<sup>22</sup> British Guinea.<sup>23</sup>

Since the recognition of the condition in Europe a number of cases of nervous complications following vaccination have been reported in this country; some of these clinically and pathologically conform to the typical European cases. Wilson and Ford,<sup>24</sup> in 1927, reported four cases from the records, the first of which occurred in 1922. In two of these cases, pathologic examination revealed lesions identical with the typical picture to be described in later paragraphs. Fulgham and Beykirch<sup>25</sup> and Tuthill<sup>26</sup> reported cases with a similar picture. Clinical cases resulting in recovery, or without satisfactory pathologic descriptions, have been reported by Graubarth,<sup>27</sup> Wolf and Brams<sup>28</sup> and Perritt and Carrell.<sup>29</sup> According to Armstrong,<sup>30</sup> possible cases,

13. Hamel: *Bull. de l'Office internat. d'hyg. pub.* **21**:2052, 1929.

14. Duken, J.: *Ztschr. f. Kinderh.* **50**:292, 1930.

15. Widowitz, Paul: *Arch. f. Kinderh.* **92**:81, 1930.

16. Kling, C.; Lonberg, N., and Wassen, E.: *Bull. de l'Office internat. d'hyg. pub.* **21**:2055, 1929.

17. Knöpfelmacher, W.: *Wien. klin. Wchnschr.* **43**:97, 1930.

18. Zappert, J.: *Wien. med. Wchnschr.* **80**:127, 1930.

19. Pagniez, P.: *Presse méd.* **38**:134, 1930.

20. Miłukowski, V.: *Schweiz. med. Wchnschr.* **58**:506, 1928.

21. Ustvedt, Y.: *Norsk. mag. f. laegevidensk.* **91**:417, 1930; abstr., *J. A. M. A.* **95**:84, 1930.

22. Doerr, R., and Breger, E., in Kolle; Krause, and Uhlenhuth: *Handbuch der pathogenen Microorganismen*, Jena, Gustav Fischer, 1930, vol. 8, p. 1531.

23. Grace, A. W.: *Tr. Roy. Soc. Trop. Med. & Hyg.* **40**:337, 1930.

24. Wilson, R. E., and Ford, F. R.: *Bull. Johns Hopkins Hosp.* **40**:337, 1927.

25. Fulgham, J. H., and Beykirch, J. G.: *J. A. M. A.* **92**:1427, 1929.

26. Tuthill, Ruth: *Arch. Neurol. & Psychiat.* **24**:759, 1930.

27. Graubarth, Julian: *Arch. Pediat.* **46**:701, 1929.

28. Wolf, Henry; and Brams, W. A.: *J. Nerv. & Ment. Dis.* **71**:714, 1930.

29. Perritt, R. A., and Carrell, R. C.: *J. A. M. A.* **94**:793, 1930.

30. Armstrong, Charles: *Pub. Health Rep.* **44**:2041, 1929.

based on clinical and epidemiologic grounds, have been noted in eight states. No cases have as yet been reported in Canada.<sup>31</sup>

A number of attempts have been made to show the presence of this complication of vaccination previous to the recent recognized outbreaks. Comby<sup>32</sup> and Knöpfelmacher<sup>33</sup> recalled similar cases seen 20 years ago. Doerr and Breger<sup>22</sup> referred to Freund as having mentioned the possibility of this complication in 1897. Jorge<sup>34</sup> recalled two cases diagnosed as tuberculous meningitis reported by Carrier, and thinks it probable that accidents of this character have been happening for many years. According to Turnbull,<sup>35</sup> Sacco, 100 years ago, referred to nervous accidents consequent on vaccination. The Royal Commission on Vaccination of the years from 1880 to 1896 made no allusion to cerebral complications.<sup>3</sup> It seems possible, however, that occasional cases have occurred since the introduction of vaccination.

In any consideration of the etiology of these reported postvaccinial complications involving the central nervous system, the first explanation that presents itself is that these cases are merely coincidental nervous diseases that have no connection with vaccination. In view of the enormous numbers of vaccinations performed annually and the comparatively few cases reported, this explanation might appear to be correct. Preparatory to a discussion of the evidence against this chance relationship and of the other theories regarding the etiology, a review of the clinical picture, epidemiology and morbid anatomy of the complications will first be given. A group of possibly related conditions, knowledge of which is essential in a discussion of the etiology of postvaccinial encephalomyelitis, will be briefly reviewed.

#### CLINICAL PICTURE

In reviewing the first large group of English cases, the Andrewes committee<sup>3</sup> considered forty-seven of the sixty-two cases sufficiently similar clinically to be regarded as a "homogeneous" group. The picture in this group they described as follows: "In most instances the onset of symptoms was rapid and the course of the disease acute. The predominant symptoms were of cerebral rather than spinal origin and included fever, headache, vomiting, strabismus and varying degrees of clouding of consciousness. Where paralysis of the limbs occurred, it was generally of the upper motor neurone type." They regarded the symptomatology as indicating a diffuse inflammation of the brain without special localization and with little evidence of involvement of the spinal cord. The Rolleston committee<sup>3</sup> considered a total of forty more cases, twenty-five of which belonged in a homogeneous group presenting clinical features similar to those

31. Defries, R. D., and McKinnon, N. E.: *Canad. M. A. J.* **21**:516, 1929.

32. Comby, J.: *Arch. de méd. d'enf.* **10**:598, 1907.

33. Knöpfelmacher: *Wien. klin. Wchnschr.* **43**:97, 1930.

34. Jorge, R.: *Bull. de l'Office internat. d'hyg. pub.* **19**:37, 1927.

35. Turnbull, H. M.: *Lancet* **2**:43, 1929.

described by the Andrewes committee. In the later report it was emphasized that the cardinal symptoms—headache, vomiting, drowsiness and pyrexia—are constantly present in severe, and rarely absent in, mild cases. They may be the only symptoms present even in fatal cases.

The continental cases seem to have been somewhat more variable in their clinical aspects. Lucksch<sup>36</sup> classified the cases according to the clinically apparent localization of the lesions. In his first group, the symptoms pointed to an involvement of the meninges alone, with headache, vomiting, fever, opisthotonos, convulsions and increased pressure of the cerebrospinal fluid. In the second group, the brain itself was involved, as well as the meninges, with paresis and somnolence as additional symptoms. The majority of the Dutch cases fell into this group. Tetanus-like symptoms were imposed in some and were present in Lucksch's original cases and in some of the English cases. Knöpfelmacher<sup>17</sup> suggested that many cases of so-called tetanus following vaccination may in reality have been encephalitis. In another small group of cases, the muscles of the eye were affected with squinting and abducens paralysis, and in others disturbances of the bladder and rectum pointed to involvement of the cord, as well as of the brain. Knöpfelmacher,<sup>17</sup> concurring in Lucksch's classification, would have added to the cerebral group cases of epilepsy occurring after vaccination. Lucksch's third main group is composed of cases in which the symptoms were confined to the cord, giving poliomyelitis-like pictures, but without the residual disabilities. Knöpfelmacher would have added a fourth large group in which neuritis followed vaccination and quoted Zappert and Leiner and others as having observed cases of this type.

Excluding the few neuritides, poliomyelitis-like cases and epilepsies, the cases as a whole show a considerable degree of similarity and are generally accepted as representing a definite clinical entity, although, as Lucksch emphasized, the diagnosis cannot, with certainty, be made on a clinical basis alone.

The typical condition can be differentiated clinically from epidemic encephalitis by the presence of a positive Babinski sign, the lack of residual lesions or recessions and, in most cases, by the absence of involvement of the muscles of the eyes. From poliomyelitis, it is differentiated by the gradual onset of the paralysis, when it occurs, and by the usually complete recovery.<sup>36</sup>

The mortality in the English cases was 58 per cent;<sup>3</sup> in the Dutch cases it was about 35 per cent.<sup>37</sup> Flexner<sup>38</sup> thought that the difference in mortality is accounted for by the inclusion of more mild cases in the Dutch collections than in the English. In most of the English cases in which the patients survived, recovery was complete; some evidence of mental change was present in five of the twelve cases followed for a long period. Mental deterioration or residual paralysis occurred in a small proportion of the continental cases.

36. Lucksch, Franz: *Centralbl. f. Bakteriöl.* (Abt. 1) **103**:227, 1927.

37. Terburgh, J. T.: *Nederl. tijdschr. v. geneesk.* **71**:2, 18 and 10, 1927.

38. Flexner, Simon: *J. A. M. A.* **94**:305, 1930.



## EPIDEMIOLOGY

*Period of Incubation.*—The time of onset of the nervous symptoms following vaccination has been carefully observed in a considerable number of cases. Chart 1, showing for each day after vaccination the number of cases having their onset on that day, is based on a total of 298 cases: 87 from the reports of the English committees, 123 from Terburgh's<sup>39</sup> report on the Dutch cases, 89 from Eckstein's<sup>40</sup> report on the disease in Germany and the remainder from isolated reports, including some of the cases in this country, in which a definite period of incubation was given. By far the great majority of cases (65 per cent) had their onset between the tenth and thirteenth days, and 82 per cent between the seventh and fourteenth days. The earliest day after vaccination on which an onset occurred was the second day. There

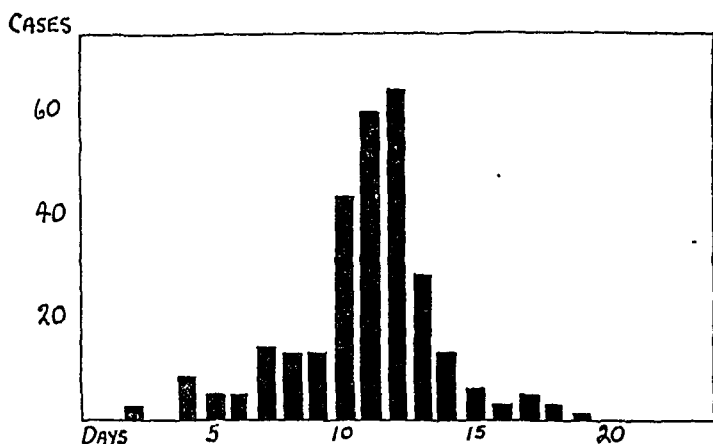


Chart 1.—Cases of postvaccinial encephalitis according to days of onset following vaccination. Twenty-one cases occurring between the twentieth and the thirty-fifth day are not shown on the chart.

is a sharp decline in the number of onsets after the thirteenth day, and only isolated cases began after the sixteenth day. The English commission found that the cases having their onsets very early or very late after vaccination were those occurring in infants. The German cases<sup>40</sup> taken alone show a greater distribution over the time chart and a much greater proportion occurring before the tenth day than is found in the total collection of cases. Chart 1 indicates a definite regularity in the time of occurrence of the disease after vaccination and justifies Keller<sup>41</sup> in speaking of a "normal" period of incubation.

*Incidence in Relation to Number of Vaccinations.*—Whether there is a definite relationship of the incidence of postvaccinial encephalitis

39. Terburgh, J. T.: Versl. med. Volksgezdh., 1929, p. 1302.

40. Eckstein, A.: Klin. Wchnschr. 8:1153, 1929.

41. Keller, W.: Monatschr. f. Kinderh. 44:222, 1929.

to the number of vaccinations performed is less certain. This proportional incidence has varied considerably in the various countries in which the disease has occurred. Possibly the highest incidence was that on the island of Marken,<sup>42</sup> where three of thirty-six vaccinated persons contracted encephalitis. The accompanying table indicates the great variability of the incidence expressed in number of vaccinations giving rise to one case.

Within the countries concerned, a somewhat closer relationship is shown, although discrepancies exist. The Andrewes Committee<sup>3</sup> made a careful study of the incidence in 1922 and 1923, in relation to the number of tubes of vaccine lymph issued each week during this period. Chart 2 is adopted from their report. The black columns in the upper part of the chart represent the postvaccinial cases of encephalitis accord-

TABLE 1.—*Incidence of Encephalitis Following Vaccination*

Place*	Author	Incidence in Relation to Number of Vaccinations
Island of Marken.....	Aldershoff.....	1 in 12
Holland, 1924-1927.....	Jitta.....	1 in 6,938
Holland, 1927.....	Jitta.....	1 in 3,074
Holland, 1928.....	Jitta.....	1 in 4,545
Sweden, 1924-1928.....	Kling, Lonberg and Wassen.	1 in 21,275
England.....	Bedson.....	1 in 48,823
Germany.....	Doerr and Breger.....	1 in 100,000
Switzerland.....	Sobernheim.....	1 in 333,000

\* In the area of Paris in six years there have been 1,324,082 vaccinations with no cases (Bull. de l'Office internat. d'hyg. pub. 21: 1133, 1929), and in Soviet Russia where there are from 8,000,000 to 9,000,000 vaccinations a year, no case of encephalomyelitis has yet been reported.

ing to the weeks in which the patients were vaccinated; those in the lower part, the tubes of lymph issued each week. The committee's conclusion that "there is undoubtedly an association in time between cases and the prevalence of vaccination throughout the country" seems justified by the chart. The relationship, however, is not a direct one, since a considerably smaller number of vaccinations performed in the summer of 1923 were followed by more cases than the greater number of vaccinations in 1922. The mortality in the cases in 1923 is not sufficiently lower to ascribe this difference in number to a greater recognition of milder cases as a result of more widespread knowledge of the condition.

In the different districts within England the relationship is not direct. The committee found that increased vaccination was accompanied by encephalitis in twenty-seven localities, but that in seven

42. Aldershoff, H., quoted by Doerr and Breger (footnote 22).

places encephalitis occurred without any increase in vaccination, and that in eighteen places increased vaccination did not result in any cases of encephalitis.

In other countries a more or less regular relationship between number of cases and number of vaccinations has been established. Hamel<sup>13</sup> and Eckstein<sup>40</sup> found that in Germany most of the cases occurred in May and June, the months when the most vaccinations were performed. In Holland from January to June, 1929, few vaccinations were performed, and only 5 cases of encephalitis were reported, but following this period, owing to an epidemic of smallpox, vaccinations were done in great numbers, and 68 cases resulted.<sup>43</sup> Terburgh<sup>39</sup> showed that over a period of three years in Holland 188,000 vaccinations were performed in March, with 46 cases; 99,000 in April, with

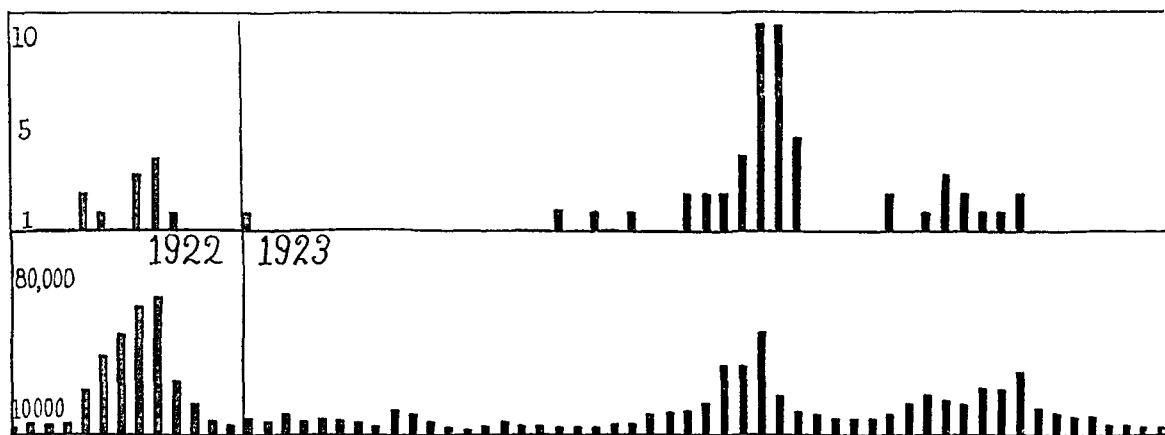


Chart 2.—Above: Cases of postvaccinal encephalitis in England from October, 1922, to December, 1923, according to the weeks when the patients were vaccinated. Below: Tubes of lymph issued each week during the same period. The chart is adopted from the Report of the Andrewes and Rolleston Committees on Vaccination, of the Ministry of Health, London, published by His Majesty's Stationery Office in 1928.

14 cases, and 46,000 in May, with 2 cases, indicating a rough proportionality of cases to vaccinations. As shown in the table, in Holland the incidence with relation to vaccinations varied from 1 in 6,938 from 1924 to 1927, to 1 in 3,074 in 1927. It may be concluded that a definite relationship exists between the number of vaccinations and the number of cases of encephalitis, but that this relationship is by no means directly proportional, and that within certain districts it is not evident.

*Grouping of Cases.*—Closely related to the lack of a directly proportional relationship between the cases of postvaccinal encephalitis and

43. Jitta, N. M. J.: Bull. de l'Office internat. d'hyg. pub. **21**:1886, 1929.

vaccinations is the so-called "group arrangement" of cases which has been noticed in many outbreaks of the disease. The English committees noticed that while many cases of the disease were isolated and sporadic, there was a tendency for groups of cases to occur closely associated in time and place, but with no evidence of a spread of infection, since the cases occurred almost simultaneously. They described the bulk of cases in July, 1923, especially, as "a simultaneous series of explosions apparently unconnected." The groups of cases were never very large—one of five cases was the largest—but they were conspicuous in contrast with the absence of cases elsewhere. The second report on the twenty-five cases in 1926 and 1927 also showed the occurrence of two definite groups of five and six cases. In Holland, nineteen of the first thirty-five cases occurred in groups of two or more in small communities. Doerr and Breger<sup>22</sup> emphasized the "high proportion of cases in a certain place at a certain time while the neighboring regions remained free." Gildemeister<sup>44</sup> mentioned three such outbreaks in Holland. Especially illustrative of this characteristic grouping were the eleven cases in the Kufstein region of the Tyrol<sup>45</sup> and the cases on the Island of Marken reported by Aldershoff.<sup>42</sup>

Grouping of cases of postvaccinial encephalitis in the same household or family has occurred, but comparatively rarely. The English committee reported two such instances, in each of which members of the family were vaccinated on the same day and showed symptoms on the same day. Two instances of more than one case occurring in the same family, but with two and three years intervening, are on record,<sup>44</sup> suggesting a peculiar familial susceptibility.

#### RURAL DISTRIBUTION

With the exception of the first cases in London in 1922, the English cases had a distinctly rural distribution; the explosive outbreaks occurred almost entirely in rural communities. The Rolleston committee<sup>3</sup> regarded a distribution among small hamlets and the very occasional incidence of familial cases as characteristic of postvaccinial encephalitis as of poliomyelitis and epidemic encephalitis.

The primarily rural distribution of the disease in Holland in the earlier cases, at least, is evident from figures calculated from the report of the Commission of Vaccinations of the League of Nations. From 1924 to 1927, the incidence per million of inhabitants in the communes, according to population was as follows: over 100,000, 13.5; from 20,000 to 100,000, 12.1; from 5,000 to 20,000, 14.7; under 5,000, 33.1. It seems likely, however, that more recently the rural predominance has decreased, for Jitta, in 1930,<sup>11</sup> stated that there is slight difference between the urban and rural incidence.

The rural distribution has not been evident in Germany. Eckstein<sup>40</sup> stated that there had been a slight preponderance of cases in the larger cities.

44. Gildemeister, E.: *Centralbl. f. Bakteriöl. (Abt. 1)* **110** (supp.): 121, 1929.

45. Kaiser, M., and Liedl, E.: *Ztschr. f. Desinfekt. u. Gesundhw.* **21**:261, 1929.

*Incidence Considered in Relation to Strain of Virus Used, and in Relation to Reaction at Site of Vaccination.*—The occurrence of postvaccinial encephalitis in isolated small outbreaks suggests that certain strains or lots of virus may be responsible, but there is no evidence incriminating any strain or lot. Most of the English cases followed use of the government lymph, but the tubes involved were from many different series, and cases also followed the use of lymph from commercial sources in England and on the continent. In Holland lymphs from many sources have been used, and no one source has proved to be more associated with the occurrence of the disease than another.<sup>10</sup> It is generally accepted that the intensity or character of the reaction at the site of vaccination bears no relation to the occurrence of the disease, the reaction running a normal course in the great majority of the cases.

*Incidence in Relation to That of Other Nervous Diseases.*—The possible relationship of the incidence of postvaccinial encephalitis to the incidence of other epidemic nervous diseases is of some importance in determining the causal factor. The similarity in epidemiology between postvaccinial encephalitis, poliomyelitis and epidemic encephalitis has been referred to. The English committee found no association of the postvaccinial disease with epidemic encephalitis, but noted an association with poliomyelitis in 1923. The majority of the postvaccinial cases occurred just previous to the seasonal rise in poliomyelitis or in the autumn when poliomyelitis was still prevalent. The cases in 1922 occurred when the incidence of poliomyelitis was still greater than normal. The same relationship was not evident in 1926 and 1927, and a study of the largest group at this time showed no unusual incidence at the time of, or in the same place with, any other nervous disease. In Holland the early cases accompanied a rise in the number of cases of epidemic encephalitis, but later the number of the latter declined, while the number of postvaccinial cases increased.<sup>22</sup> There was no relation between the incidence of postvaccinial cases and that of poliomyelitis in Holland. In Bohemia, Lucksch<sup>4</sup> was unable to find any relationship between the occurrence of his cases and the occurrence of poliomyelitis or of encephalitis. In Germany, according to Doerr and Breger,<sup>22</sup> epidemic encephalitis had declined before postvaccinial encephalitis occurred. They pointed out that in France and the United States epidemic encephalitis is common, while postvaccinial encephalitis is rare. It is questionable whether any importance is to be attached to the apparent relationships to poliomyelitis in England and epidemic encephalitis in Holland. The apparent general increase in other post-infection and independent encephalitides coincidental with an increase in postvaccinial encephalitis, will be discussed in another section.

*Incidence in Relation to Age.*—The determination of a predilection in postvaccinal encephalitis for any special age is complicated by the differences in the laws regulating vaccination in the various countries. The frequencies according to age must be expressed in relation to the number of vaccinations in any one age group. However, from the various reports, it seems definite that infants have a relative insusceptibility to the disease. In England,<sup>3</sup> where 63.3 per cent of the vaccinations are performed on children under 1 year of age, it was shown that of ninety-three cases, fifty-nine should have been in children under 1 year of age, but that only nine of the cases were. The occurrence of cases in England was associated with an increased proportion of vaccinations later than infancy, owing to the increasing neglect of vaccination. A similar insusceptibility of infants was noticed in Holland,<sup>10</sup> where the ratio of cases to vaccinations in children under 2 years of age was 1:13,531, while in children from 3 to 12 years of age the ratio was 1:3,555. In Germany no particular distribution with relation to age was noticed by Eckstein<sup>40</sup> or by Hamel,<sup>13</sup> most cases occurring in the groups from 1 to 2 years of age on which the most vaccinations had been performed. But Knöpfelmacher pointed out that in four large cities of Germany, 28 per cent of the vaccinations, as opposed to 13 per cent of the cases of postvaccinal encephalitis, were in children under 1 year of age, while 50 per cent of the vaccinations and 40 per cent of the cases of postvaccinal encephalitis occurred in children in their second year of age, apparently showing an increase in susceptibility in the second year. Of the cases in Vienna and lower Austria, none occurred in children under 3 years of age, although one third of the vaccinations were performed on children under 2.<sup>18</sup>

From these or similar figures a number of authors have concluded that children of school ages are relatively very susceptible to the disease. From the figures at my disposal, no comparison other than that of early infancy with other ages can be made. Whether adults are more or less susceptible than school children cannot be determined, but that young infants are definitely less susceptible seems to be established.

A number of cases have been reported in which children were ill just previous to, or at the time of, vaccination, which suggests that a general lessening of resistance might be a factor in the occurrence of the complication. Coyl and Hurst<sup>46</sup> and Horder<sup>47</sup> reported cases occurring in children vaccinated while convalescing from pneumonia, or while still weak from infectious disease. It is, of course, possible that these cases were coincidental, since it is probable that many children are vaccinated under similar conditions with no evil effects.

There is no indication of any seasonal occurrence, the time of appearance apparently depending on the seasonal variations in vaccinations. The great majority of cases have occurred in northern Europe, but a number have been reported in Italy,<sup>22</sup> and at least one case has occurred in the tropics.<sup>23</sup>

#### MORBID ANATOMY

That the pathologic picture presented by the postvaccinal cases of encephalitis is constant and typical and can be definitely differentiated from that of poliomyelitis and that of epidemic encephalitis is

46. Coyl, C. D., and Hurst, E. W.: *Lancet* 2:1246, 1929.

47. Horder, Thomas: *Lancet* 1:1301, 1929.

accepted by most investigators. Turnbull<sup>48</sup> gave a complete résumé of his own observations and those of McIntosh<sup>2</sup> and correlated them with the descriptions of Bastiaanse<sup>5</sup> (1925), Perdrau<sup>49</sup> (1928), Schurman<sup>50</sup> (1928) and Lucksch<sup>36</sup> (1927). The distribution of the lesions is typical and constant. Focal changes occur throughout the central nervous system from cortex to lumbosacral cord. The white and gray matter are both extensively involved. The meninges are only slightly affected, the characteristic changes occurring within the central nervous system. The essential lesions are perivascular and marginal zones of demyelination and perivascular infiltration, largely extra-adventitial. The demyelination is almost always around veins and extends for long distances along their branches; it is usually complete, only a fraction of the axis cylinders surviving in any zone. In the acute cases a great number of proliferated glial cells are usually contained in the wide-meshed net of glial fibrils in the zones of demyelination. Proliferated glial cells extend a variable distance beyond the zones of demyelination and cause a diffuse infiltration in the most severely affected regions. Hemorrhages are rare and, when they occur, are small and perivascular. Tigrolysis in the nerve cells is constant, but complete necrosis is rare, and neuronophagia is seldom, if ever, seen.

McIntosh and Scarff<sup>51</sup> had a different conception of the essential lesions, which becomes important later in the comparison of experimental vaccinia encephalitis in animals with the postvaccinia disease. They considered the essentially characteristic feature to be the radiating infiltration of parenchyma by large endothelial cells with clear, oval nuclei. These cells occur in an apparent radiating growth out from the central mass and give an appearance like that of a tissue culture. The demyelination was considered by McIntosh to be of secondary importance. Coyl and Hurst<sup>46</sup> and Taylor<sup>52</sup> reported cases conforming to the foregoing general description from Turnbull. Taylor considered the infiltration to be made up chiefly of endothelial cells. In this country, the cases of Wilson and Ford,<sup>24</sup> Fulgham and Beykirch (studied by Flexner<sup>38</sup>) and Tuthill<sup>26</sup> conform to the same general picture.

#### POSSIBLY RELATED CONDITIONS

There are a number of pathologic conditions occurring independently or as complications of other diseases which on clinical or pathologic grounds have been regarded as identical with, or closely related to,

48. Turnbull, H. M.: *Brit. M. J.* **2**:331, 1928.

49. Perdrau, J. R.: *J. Path. & Bact.* **31**:17, 1928.

50. Schurman, Paul: *Beitr. z. path. Anat. u. z. allg. Path.* **79**:409, 1928.

51. McIntosh, J., and Scarff, R. W.: *Proc. Roy. Soc. Med.* **21**:705, 1928.

52. Taylor, J. F.: *Lancet* **1**:1302, 1929.

the encephalomyelitis following vaccination. Smallpox, measles, chickenpox, German measles, mumps, scarlatina, whooping cough, typhoid fever, typhus fever, yellow fever and psittacosis all have been shown to include lesions of the central nervous system, either as an integral part of the disease or as an occasional complication. Conditions in which the symptoms or lesions were possibly very similar to those of the postvaccinial diseases have occurred following antirabic treatment and also as apparent clinical entities independent of any known disease as an exciting factor. Greenfield<sup>53</sup> took over the term "acute disseminated encephalomyelitis" from Westphal to describe a group of these conditions which he regarded as proved on pathologic grounds to be very similar. He included in this group only conditions following vaccinia, variola and measles, and some independent forms. He considered that there is no satisfactory evidence that the pathologic picture in the other cases is the same.

*Encephalomyelitis Complicating Smallpox.*—Aldrich,<sup>54</sup> in 1904, collected from the literature the reports of cases of smallpox in which there was involvement of the central nervous system and added several previously unrecorded. He stated that cases of smallpox complicated by paralysis have been recorded since the disease passed from tradition to record. The occurrence of postvaccinial encephalitis has aroused interest in these cases, and adequate summaries of the literature have been given by Turnbull and McIntosh,<sup>2</sup> Wilson and Ford,<sup>24</sup> and Troup and Hurst.<sup>55</sup> Troup and Hurst studied the records of mortality from smallpox in England and Wales from 1925 to 1929. Of 140 deaths in 51,243 cases of smallpox, 23 were ascribed to conditions of the central nervous system. The diagnoses in six of these cases indicated that the condition was encephalitis. Schamberg and Kolmer,<sup>56</sup> among the records of 3,000 cases of smallpox, found 8 in which there was mention of paralysis, and Rolleston,<sup>57</sup> among the records of 10,000 in the London epidemic of 1921 and 1922, found only 25 in which there was note of nervous complications.

On clinical grounds the cases have been divided into several groups according to the area of the central nervous system involved. Apparently the most frequent type is that in which paralysis of the legs without other symptoms occurs. The paralysis is usually flaccid, and muscular wasting and loss of reflexes occur. The

53. Greenfield, J. G.: *A System of Bacteriology*, London, Medical Research Council, 1930, vol. 7, p. 133.

54. Aldrich, C. J.: *Am. J. M. Sc.* **127**:198, 1904.

55. Troup, A. G., and Hurst, E. W.: *Lancet* **1**:566, 1930.

56. Schamberg, J. F., and Kolmer, J. A.: *Acute Infectious Diseases*, ed. 2, Philadelphia, Lea & Febiger, 1928, p. 241.

57. Rolleston, J. D.: *Acute Infectious Diseases*, London, Heinemann, 1929, p. 379.



symptoms may be entirely bulbar, with dysarthria and disturbances of swallowing, or these symptoms may be combined with spastic weakness, ataxia and sometimes mental changes, indicating a more diffuse involvement resembling disseminated sclerosis. According to Troup and Hurst,<sup>55</sup> the mode of onset may suggest transverse myelitis, and only later a certain degree of weakness of the arms and drowsiness indicate involvement of the upper part of the central nervous system. The picture in this later extension differs from that in the postvaccinal cases, in which as a rule there is no progression after the first symptoms. In the great majority of variolar complications, the nervous symptoms appear before the sixteenth day of the disease, and in some cases, even before the eruption appears. Partial or complete recovery is the rule, but permanent disturbances are more frequent than with the postvaccinal condition. That the complications are not due to especially neurotropic strains of virus is indicated by the report of Troup and Hurst<sup>55</sup> in which a case with, and fifteen cases without, nervous complications were contracted from the same source of infection.

McIntosh,<sup>58</sup> McIntosh and Scarff,<sup>51</sup> and Troup and Hurst<sup>55</sup> found that the pathologic picture in the cases reported by them could not be differentiated from that described as characteristic of the postvaccinal cases. McIntosh found the tissue-culture-like arrangement of endothelial cells which he described as occurring in postvaccinal encephalitis even more pronounced in encephalitis of variolar origin.

*Encephalomyelitis Complicating Measles.*—Comprehensive reviews of the literature on the occurrence of nervous complications of measles are contained in papers by Ford<sup>59</sup> (1928) and Greenfield<sup>60</sup> (1929). Greenfield emphasized the fluctuation in the incidence of these complications. Sydenham, in his description of the epidemics of 1670 and 1674, made no mention of nervous sequelae, but cases were described by Lucas (1790), Abercrombie (1845) and Barlow and Penrose (1886), and a small outbreak occurred in London in 1904 and 1905. In recent years, since 1924, there has been another period of increased incidence, with cases occurring on the continent and in America. Reports of individual cases from England, France and Germany in the interval indicate that the disease had not disappeared entirely during the intervening years. Boenheim,<sup>61</sup> summarizing the observations in 5,940 cases of measles in the Berlin hospitals from 1905 to 1925, found the incidence of nervous complications to be 4 per thousand cases.

Clinically, the cases are grouped according to the area of apparent involvement. There may be only cerebral symptoms of brief duration from which recovery is complete, or these may be combined with neurologic disturbances that are not revealed until the cerebral phase passes. Paraplegias indicating involvement of

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58. McIntosh, James: Brit. M. J. **2**:334, 1928.

59. Ford, F. R.: Bull. Johns Hopkins Hosp. **43**:140, 1928.

60. Greenfield, J. G.: Brain **52**:171, 1929.

61. Boenheim, Curt: Ergebn. d. inn. Med. u. Kinderh. **28**:598, 1925.

the spinal cord may occur alone or in conjunction with cerebral symptoms, indicating a general involvement of the central nervous system. Ataxias indicating cerebellar involvement are common. The onset of the nervous symptoms almost always follows the fever-free interval of, from one to two days after the rash is out, that is, about the seventh day of the disease, but it may occur before or at the height of the eruption or may be delayed for from two to three weeks.

The recent descriptions of the microscopic changes are in general agreement. From Wohlwill's descriptions of two cases of measles Turnbull<sup>48</sup> regarded the involvement of the central nervous system in these cases as practically identical pathologically with that in the postvaccinial disease. Greenfield<sup>60</sup> regarded the picture as similar to, if not identical with, that of the postvaccinial complication. The differences lie in the greater amount of perivascular infiltration and the more complete demyelination in the postvaccinial forms. The distribution of the zones of demyelination along the margins of the cord and the walls of the ventricles is the same in both conditions. Ford<sup>59</sup> also found a much smaller amount of perivascular infiltration in his cases of measles. Zimmerman and Yannet<sup>62</sup> found little involvement of the gray matter, in contrast with that in the generally accepted picture of the postvaccinial type, in which both gray and white matter are usually extensively involved.

*Nervous Complications of Varicella.*—Winnicott and Gibbs<sup>63</sup> and Wilson and Ford<sup>24</sup> published summaries of the literature dealing with the nervous complications of chickenpox, with descriptions of cases seen by themselves. More recent observers (Conrad,<sup>64</sup> Bertoye and Garcin,<sup>65</sup> Graham<sup>66</sup>) have added reports of five cases to the round dozen collected by the aforementioned authors. The symptoms are somewhat variable, but can again be classified as indicating involvement of the brain, the brain stem or the spinal cord, or all three. The time of onset of the complications is rather constant, occurring chiefly from the ninth to the thirteenth day of the varicella, but sometimes as early as the fourth or as late as the fifteenth day. Recovery always occurs and is probably invariably complete. Since no autopsies have been performed, the nature of the lesions is unknown.

*Nervous Complications of Mumps.*—The occurrence of nervous complications of mumps was first noted by Hamilton in 1758. Reports of cases and comprehensive summaries of the literature were published

62. Zimmerman, H. M., and Yannet, Herman: Arch. Neurol. & Psychiat. **24**:1000, 1930.

63. Winnicott, D. W., and Gibbs, N.: Brit. J. Child. Dis. **23**:107, 1926.

64. Conrad, C. E.: Arch. Pediat. **46**:716, 1929.

65. Bertoye, P., and Garcin: Presse méd. **37**:1517, 1929.

66. Graham, Stanley: Arch. Dis. Childhood **5**:146, 1930.

by Haden<sup>67</sup> and Weissenbach, Basch and Basch.<sup>68</sup> The condition had been largely regarded as essentially a meningitis, but Haden showed that the cerebral symptoms were out of proportion to the amount of meningeal reaction, as indicated by the spinal fluid, and could also occur without meningeal signs. Weissenbach, Basch and Basch classified the cases as meningeal or meningo-encephalitic, either complicating typical mumps or occurring independently with only slight clinical or epidemiologic evidence, pointing to the etiologic factor. The onset may precede or follow the parotid swelling, but occurs most often at the height of the parotid lesion. Testicular involvement indicating a generalization of the virus is often associated. The incidence varies considerably; 16 cases have been observed in 653 cases of mumps, but over 1,000 cases of mumps without any nervous complications have been recorded. Few necropsies have been recorded; marked congestion of the brain and serous meningitis apparently were the only changes.

Experimentally, Wollstein<sup>69</sup> and Gordon<sup>70</sup> were able to produce nervous symptoms and lesions in cats and monkeys by the intrathecal and intracerebral injection of bacteria-free filtrates of saliva from patients with mumps. Sections of the brain from one of Gordon's animals showed definite infiltrative evidence of encephalitis, although pronounced degenerative changes in the nerve cells of cortex and anterior horns were the most marked feature.

*Other Postinfection Encephalitides.*—According to McIntosh,<sup>58</sup> encephalitis is a constant feature of typhus fever and may be regarded as part of the disease. The lesions, consisting essentially of proliferations of large, clear cells into the surrounding nerve tissue, with infiltration by inflammatory cells (plasma cells, lymphocytes and a few polymorphonuclears), are regarded by McIntosh as showing great similarity to the lesions in postvaccinal and postvariolar encephalitis. Cerebral complications are also said to occur in yellow fever,<sup>71</sup> and experimentally, cerebral lesions, consisting of proliferation of the capillary endothelium and perivascular infiltration by mononuclears, were produced in mice by this virus. Encephalitic syndromes have complicated or followed psittacosis (Krichefski,<sup>72</sup> Thompson<sup>73</sup>), rubella

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67. Haden, R. L.: Arch. Int. Med. **23**:737, 1919.

68. Weissenbach, R. J.; Basch, Georges, and Basch, Marianne: Ann. de med. **27**:5, 1930.

69. Wollstein, Martha: J. Exper. Med. **34**:537, 1921.

70. Gordon, M. H.: Lancet **1**:652, 1927.

71. Theiler, Max: Science **71**:367, 1930.

72. Krichefski, H. J.: Brit. M. J. **1**:1093, 1930.

73. Thompson, A. P.: Lancet **1**:396, 1930.

(Debie <sup>74</sup>), scarlatina <sup>75</sup> and pertussis (Askin and Zimmerman <sup>76</sup>). Such pathologic investigations as have been described indicate that the encephalitic complications of the two latter conditions are not related to the group of encephalitides complicating vaccination, variola and measles.

*Paralysis Complicating Antirabic Treatment.*—On the basis of the pathologic picture, the paralyzes sometimes complicating antirabic treatment have been considered by some observers to be similar to the postvaccinial disease. A considerable number of antirabic treatments (4,836) had been given before this accident was first noticed in 1887. It was regarded as a paralytic form of rabies until it occurred in a patient proved to have been bitten by a healthy animal. Stuart and Krikorian <sup>77</sup> reviewed the literature and published reports of cases and experimental work. The condition is directly related to the treatment and probably to the injection of diseased or normal brain, since similar conditions can be produced in animals by the injection of normal brain (Remlinger, <sup>78</sup> Schweinberg, <sup>79</sup> Stuart and Krikorian <sup>77</sup>). Individual predisposition of the patient is an important factor; brain-workers are more susceptible than laborers, and Europeans more than natives of the tropics. The League of Nations Conference on Rabies placed the incidence at 0.28 per thousand. The symptoms usually begin about seven days after the beginning of treatment. The peripheral nerves only may be affected, giving local paralyzes; or the symptoms may be those of subacute dorsolumbar myelitis or of acute ascending (Landry's) paralysis. Stuart and Krikorian regarded perivascular infiltration by lymphocytes and plasma cells and demyelination as the essential features. Turnbull <sup>48</sup> referred to descriptions of the condition and stated that the pathology appears to be similar to that of the postvaccinial disease, but that the descriptions are not precise.

*Independent Conditions.*—On the basis of the demyelination as the principal pathologic feature a number of independent conditions have been considered as possibly related to postvaccinial encephalitis. Pette <sup>80</sup> regarded acute disseminated sclerosis occurring spontaneously in children and possibly multiple sclerosis as being closely related to

74. Debie, Robert; Turquetz, Roger, and Broca, Robert: Presse méd. **38**:348, 1930.

75. Editorial, Lancet **1**:1191, 1930.

76. Askin, J. A., and Zimmerman, H. M.: Am. J. Dis. Child. **38**: 97, 1929; footnote 75.

77. Stuart, G., and Krikorian, K. S.: Ann. Trop. Med. **22**:327, 1928.

78. Remlinger, P.: Compt. rend. Soc. de biol. **83**:171, 1920.

79. Schweinberg, Fritz: Wien. klin. Wchnschr. **37**:797, 1924.

80. Pette, H.: Centralbl. f. Bakteriöl. (Abt. 1) **110** (supp.):134, 1929.

postvaccinial encephalitis. Multiple sclerosis is generally recognized as differing in that the demyelination is patchy, not following a blood vessel for far, while in the postvaccinial condition the demyelination extends for long distances along the affected vessel. Greenfield<sup>81</sup> considered Pette's cases on clinical (being subacute and progressive) and on pathologic grounds as being not strictly comparable. He stressed the fact that the postvaccinial encephalitis and that complicating measles do not become progressive. Greenfield himself described two cases of acute paraplegia with influenza-like onset that showed lesions similar in distribution and character to the lesions of the central nervous system in measles and postvaccinial disease. He included these cases in his group of cases of "acute disseminated encephalomyelitis." Brain and Hunter<sup>82</sup> described a group of cases with remarkable clinical similarity to those of postvaccinial disease. In the one fatal case the pathologic changes were those of toxemia and were not similar to those in postvaccinial disease. They referred to a considerable number of similar cases recorded in the literature, in many of which the condition had apparently been of mildly epidemic form.

The relationship of the conditions described to postvaccinial encephalitis is uncertain, with the possible exception of the encephalitides complicating variola and measles and the two independent cases of Greenfield, which apparently presented a similar pathologic picture and could be assumed to be due to the same or similar viruses.

#### THEORIES REGARDING THE ETIOLOGY OF POSTVACCINIAL ENCEPHALITIS

*Accidental Relationship.*—Several possible explanations have been advanced for the occurrence of encephalomyelitis complicating vaccination. While not widely held and probably conclusively disproved, the view that the condition has an accidental relationship to vaccination must first be considered. The small, irregular incidence of the disease at first suggests that the connection is coincidental, and it might be maintained that the nervous lesions are caused by some of the known encephalitogenic viruses or some other unknown factor operating in the patient, by chance, at the time of vaccination. The evidence against this view has been largely presented in the sections on epidemiology and pathology, but will be briefly restated here. Clinically, the majority of cases show a considerable degree of similarity, and the condition is accepted as a definite clinical entity which can be differentiated from epidemic encephalitis and poliomyelitis. The marked constancy of the

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81. Greenfield, J. G.: J. Path. & Bact. **33**:453, 1930.

82. Brain, R. W., and Hunter, Donald: Lancet **1**:221, 1929.

period of incubation in itself is possibly sufficient to rule out the theory of fortuitous occurrence. The definite, though not direct, relationship of incidence to the number of vaccinations performed further supports the assumption that there is an actual causal relationship between vaccination and nervous complications. Greenwood's calculations<sup>83</sup> show that the number of deaths from acute nervous disease in the vaccinated group at the time of the maximum occurrence of postvaccinial cases in England was higher than would be probable if the deaths were due to the chance occurrence of the nervous disease in the vaccinated persons. With the possible exceptions of poliomyelitis in England and epidemic encephalitis in the early Dutch cases, there has been no association of postvaccinial encephalitis with other epidemic nervous disease that might suggest an overlapping with these diseases. The constant pathologic picture not related to that of poliomyelitis or to that of epidemic encephalitis and similar only to that of the few conditions mentioned rules out the possibility of the condition being poliomyelitis or epidemic encephalitis.

While a definite causal connection between vaccination and the majority of the reported nervous disorders may be accepted, it is probable<sup>83</sup> that some of the conditions regarded as being "propter," as well as "post," vaccinial, are due merely to inaccurate diagnosis plus a fortuitous relationship. Reisch<sup>84</sup> reported an epidemic that appeared in both vaccinated and unvaccinated children which, with insufficient investigation, could have been regarded as a true postvaccinial disease, since the only fatal case was in a vaccinated child. Grey and Whitaker<sup>85</sup> performed an autopsy in a case reported clinically as postvaccinial encephalitis and found staphylococcal septicemia with multiple pyemic abscesses on the surfaces of the brain. A case cannot be finally regarded as due to vaccination unless epidemiologically, clinically and pathologically it accords with the typical cases considered in foregoing paragraphs. It is inevitable that a number of nervous diseases should manifest themselves coincident with a procedure carried out on such a large scale as vaccination.

Since it is generally admitted and probably proved that certain cases of encephalomyelitis are attributable to vaccination, there remains to be discussed the possible ways in which vaccination might cause these complications. Three hypotheses have been put forward:

1. The nervous lesions are caused by the direct action of vaccine virus on the central nervous system.

83. Gins, H. A.: *Med. Welt* **3**:1277, 1929.

84. Reisch, O.: *Wien. klin. Wchnschr.* **43**:103, 1930.

85. Grey, T. F., and Whitaker, W. M.: *Brit. M. J.* **1**:1125, 1930.

2. The nervous lesions are not caused by the vaccine virus, but by some other virus or bacterium introduced with the vaccine virus or already present, dormant, in the vaccinated person and activated by the vaccine virus or by the decrease in resistance of the patient brought about by the febrile reaction.

3. The complication is the result of an anaphylaxis-like reaction to the virus or to a nonspecific factor.

The discussion of the evidence in favor of or against these theories will be taken up in the order given.

*Vaccine Virus as the Direct Cause.*—The production of nervous symptoms and lesions in animals by certain strains of vaccine virus and the fact that many of the filtrable viruses or closely related forms can cause encephalitis in human beings or experimentally in animals are the primary bases on which this theory is founded. It was formerly considered that infection with vaccine virus was a local phenomenon, the virus growing in dermal tissue and only incidentally overflowing into the blood. It is now known that vaccine virus can be recovered from the blood and organs of animals and man for a number of days after inoculation, and that the virus will grow in many different tissues.

Marie,<sup>86</sup> in 1920, showed that vaccine virus injected intracerebrally into rabbits would kill them, giving rise to nervous symptoms, with apparent growth of the virus in the brain. Levaditi, Harvier and Nicolau,<sup>87</sup> in 1921, by a process of adaptation through alternate testicle and brain passage, repeated Marie's work and was able to produce a fixed "neurovaccine" which he could pass indefinitely in series through rabbits' brains. Many workers since then (Condrea,<sup>88</sup> Krumbach,<sup>89</sup> Blanc and Caminopetros,<sup>90</sup> von Wasielewski and Winkler,<sup>91</sup> Bachman and Biglieri,<sup>92</sup> Ledingham<sup>93</sup>) have shown that with many strains of virus, at least, no process of adaptation is necessary: that intracerebral injection of calf virus will produce symptoms and death, and that such viruses can be passed indefinitely from brain to brain. Others have had difficulty<sup>58</sup> in adapting, or have been entirely unable to adapt,

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86. Marie, A.: *Compt. rend. Soc. de biol.* **83**:476, 1920.

87. Levaditi, C.; Harvier, P., and Nicolau, S.: *Compt. rend. Soc. de biol.* **85**:345, 1921.

88. Condrea, P.: *Compt. rend. Soc. de biol.* **86**:897, 1922.

89. Krumbach, H.: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **38**:1, 1923.

90. Blanc, Georges; and Caminopetros, J.: *Compt. rend. Soc. de biol.* **88**:1020, 1923.

91. von Wasielewski, T., and Winkler, W. F.: *Ergebn. d. Hyg., Bakt., Immunitätsforsch. u. exper. Therap.* **7**:1, 1925.

92. Bachman, A., and Biglieri, R.: *Compt. rend. Soc. de biol.* **88**:351, 1923.

93. Ledingham, J. G. G.: *J. State Med.* **34**:1925, 1926.

some strains with which they were working (Camus,<sup>94</sup> Cattaneo,<sup>95</sup> Thompson<sup>96</sup>). I<sup>96</sup> was unable to adapt to the brain either the Noguchi strain of testicular virus or the New York City Board of Health strain from which the former was derived. But a commercial strain, a descendant of the same New York virus, easily, on first injection and on subsequent passages, produced nervous symptoms and death and multiplied profusely in the brain. Levaditi, Nicolau and Sanchis-Bayarri,<sup>97</sup> and Aldershoff, Pondman and Pot,<sup>98</sup> produced meningo-encephalitis in monkeys by the intracerebral injection of virus. The reaction of the skin to vaccine virus grown in brain (so-called neurovaccine) is characteristically more violent and definitely more hemorrhagic than that to ordinary strains of virus (Camus,<sup>94</sup> Aldershoff, Pondman and Pot,<sup>98</sup> Levaditi,<sup>99</sup> Burnet and Conseil,<sup>100</sup> and in my own experience). Neurovaccine is considered by Levaditi<sup>99</sup> to be more neurotropic (to settle more readily in the brain after peripheral inoculation) than other strains, but others have shown that any strain of vaccine virus can often be found in the brain after peripheral inoculation (Blanc and Caminopetros,<sup>90</sup> Huon and Placidi,<sup>101</sup> Barikine, Kompaneetz, Zakharoff and Barikine,<sup>102</sup> Hach,<sup>103</sup> Minervin and Schmerling<sup>104</sup>). In attempting to produce encephalitis in rabbits by peripheral inoculation, many workers have had negative results (Condrea,<sup>88</sup> Cattaneo,<sup>95</sup> Demme,<sup>105</sup> Walthard,<sup>106</sup> Olitsky and Long<sup>107</sup>), but some were occasionally successful (Bachman and Biglieri,<sup>92</sup> Levaditi,<sup>99</sup> Huon and Placidi,<sup>101</sup> Rhoades,<sup>108</sup> McIntosh and Scarff,<sup>109</sup> Hoffmann<sup>110</sup>). Clear-

94. Camus, M. L.: *Bull. Acad. de méd., Paris* **90**:79, 1923.

95. Cattaneo, L.: *Policlinico (sez. prat.)* **35**:759, 1928.

96. Thompson, Richard: *Proc. Soc. Exper. Biol. & Med.* **26**:559, 1929.

97. Levaditi, C.; Nicolau, S., and Sanchis-Bayarri, V.: *Presse méd.* **35**:161, 1927.

98. Aldershoff, A.; Pondman, A. B., and Pot, A. W.: *Ann. Inst. Pasteur* **43**:1268, 1929.

99. Levaditi, C.: *J. State Med.* **32**:151, 1924.

100. Burnet, E., and Conseil, E.: *Compt. rend. Soc. de biol.* **90**:1408, 1924.

101. Huon and Placidi: *Compt. rend. Soc. de biol.* **91**:308, 1924.

102. Barikine, W.; Kompaneetz, A.; Zakharoff, A., and Barikine, O.: *Compt. rend. Soc. de biol.* **90**:1134, 1924.

103. Hach, I. W.: *Ztschr. f. Hyg. u. Infektionskr.* **104**:569, 1925.

104. Minervin, S., and Schmerling, A.: *Centralbl. f. Bakteriöl. (Abt. 1)* **99**:558, 1926.

105. Demme, Hans: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **55**:191, 1928.

106. Walthard, B.: *Schweiz. med. Wchnschr.* **56**:854, 1926.

107. Olitsky, P. K., and Long, P. H.: *J. Exper. Med.* **48**:379, 1928.

108. Rhoades, C. P., quoted by Flexner (footnote 38).

109. McIntosh, J., and Scarff, R. W.: *J. Path. & Bact.* **33**:483, 1930.

110. Hoffmann, D. C.: *J. Exper. Med.* **53**:43, 1931.



kin<sup>111</sup> succeeded in producing symptoms and lesions of the brain by intradermal inoculation of vaccine virus into wild African monkeys.

In view of the peculiar characteristics of the lesions of the skin produced by neurovaccine, Camus<sup>94</sup> suggested that its ability to produce cerebral lesions may be due to its admixture with some contaminating virus. The apparent impossibility of adapting certain strains of vaccine virus to the brain might be interpreted as supporting this conception. Lack of immunity relationships between neurovaccine and herpes virus<sup>112</sup> indicates that the possible contaminant cannot be herpes virus. Addition of a poliomyelitic virus to a nonencephalitogenic strain of vaccine virus did not render this strain encephalitogenic for rabbits,<sup>113</sup> suggesting that poliomyelitic virus is not the supposed additional virus. All attempts, by passage through animals immune to a nonencephalitogenic strain of vaccine virus, to separate from neurovaccine some nonvaccinal factor which, when added to the nonencephalitogenic virus, would produce a neurovaccine have been unsuccessful.<sup>114</sup> All the evidence at one's disposal indicates that the neurovaccines are not contaminated viruses, but vaccine viruses that have acquired the ability to grow in nervous tissue. This change cannot always be produced at will, but may occur spontaneously, as in the commercial strain of virus referred to. It is possibly associated with an increase in generalizing power, but not necessarily in virulence for the skin as measured by titration by dilution.

The evidence supporting the hypothesis that the human postvaccinial condition is an expression of this encephalitogenic power of vaccine virus which has been described will now be considered.

**Epidemiologic Evidence:** The constancy of the relationship between the onset of symptoms and the time of vaccination which has been stressed is regarded by the proponents of the theory that vaccine virus is the direct cause as indicating the vaccinal nature of the nervous lesions. There are, however, other possible explanations of the constant period of incubation. An activated virus introduced at the time of vaccination would also probably be characterized by a fairly regular period of incubation. However, all attempts to show the presence of any known virus in the lymph used have failed, although Perdrau<sup>115</sup> showed that herpes and vaccinia could be carried along together in animal passage. The lack of evidence incriminating any particular batch or batches of lymph also indicates that the encephalitis is not due to contamination of the lymph. It is possible<sup>3</sup> that the so-called constancy of the period of incubation may be more apparent than real, owing to the fact that the constitutional symptoms present just

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111. Clearkin, P. A.: *Brit. J. Exper. Path.* **11**:329, 1930.

112. Holden, M., and Thompson, Richard: Unpublished work; Olitsky and Long (footnote 107).

113. Thompson, Richard: *J. Exper. Med.* **51**:777, 1930.

114. Thompson, Richard; and Buchbinder, Leon: Unpublished work.

115. Perdrau, J. R. (footnote 3).

before the reaction to the vaccination reaches a maximum may call attention to cerebral symptoms for the first time.

The similarity of the periods of incubation in postvaccinial encephalitis, variola and generalized vaccinia in man has been considered as evidence in favor of the vaccinal nature of the postvaccinial disease.<sup>44</sup> It is regarded as probable that the mechanism is the same in each case; a local multiplication of the virus with secondary generalization producing the general disease in smallpox, the lesions of the skin in generalized vaccinia and the changes in the central nervous system in postvaccinial encephalitis. The fact that the generalization of the virus, as indicated by the positive results from examination of the blood, is probably at its height at about the same time also favors this view (McIntosh<sup>116</sup>).

Netter<sup>117</sup> stated that the disease occurs after revaccination in a much smaller percentage of cases than after primary vaccination and regards this as evidence favoring the theory that vaccine virus is the direct cause, since the revaccinated persons have a degree of vaccinal immunity. Statistical data comparing the incidence of the complication in the two groups are not available, but the general impression seems to support Netter's views as to the comparatively rare occurrence in revaccinated persons. The information regarding the occurrence of a previously successful "take" is not always reliable, but there have been a number of cases reported as following probably authenticated revaccination (Hamel,<sup>118</sup> Mader,<sup>118</sup> Rosendahl,<sup>119</sup> Hekman<sup>120</sup>). Hekman<sup>120</sup> reported a number of cases following revaccination and stated that in these cases the period of incubation was shorter than in cases following primary vaccination. He regarded this as further evidence of the vaccinal nature of the disease, comparing the acceleration to the shortening of the period of incubation in varioloid and vaccinoid. It is indeed difficult to explain the shorter period of incubation on any other basis, but here again one lacks conclusive statistical evidence of an accelerated process. It would be interesting to determine whether more cases have followed revaccination in Germany than in other countries, accounting for the shorter average period of incubation in that country.

Isolation of Vaccine Virus from the Nervous System in Cases of Postvaccinial Encephalitis: The results of experimental and pathologic

116. McIntosh, James: *Lancet* **1**:618, 1930.

117. Netter, A., quoted by Paris correspondent: *Vaccinal Encephalitis*, *J. A. M. A.* **93**:2040, 1929.

118. Mader, A.: *Jahrb. f. Kinderh.* **123**:111, 1929.

119. Rosendahl, H. M.: *Nederl. tijdschr. v. geneesk.* **73**:5117, 1929; abstr., *Bull. Hyg.* **5**:236, 1930.

120. Hekman, M. J.: *Tribuna med.* **64**:307, 1930.

investigation of postvaccinial encephalitis have been used by all the proponents of theories to support their respective views. A crucial point in favor of the theory the vaccine virus is the direct cause would be the presence of vaccinia virus in considerable amounts in the brain in fatal cases, since in rabbits, when encephalitis occurs, there is a profuse increase of virus in the brain. While a small number of workers have reported the presence of vaccine virus in the brain in fatal cases (Turnbull and McIntosh,<sup>2</sup> McIntosh and Blaxall,<sup>121</sup> Aldershoff,<sup>122</sup> Bijl<sup>123</sup>), it has usually been in small amounts and detected only after enrichment by passage through the testicle. Many workers have had entirely negative results (Luksch,<sup>4</sup> Perdrau,<sup>115</sup> Fildes,<sup>124</sup> Bastiaanse, Bijl and Terburgh,<sup>125</sup> Levaditi, Lepine and Troisier,<sup>126</sup> Maitland,<sup>127</sup>). While the supporters of the theory that the disease is due to activation of another virus regard the presence of the virus in the brain in small amounts as a probable concomitant of normal vaccination, the supporters of the theory that vaccine virus is the direct cause would explain its absence, in those cases in which it is not found, on the basis of autosterilization as expounded by Levaditi to explain the absence of herpes virus from the brain in cases of epidemic encephalitis (Netter<sup>128</sup>). Bijl and Fraenkel<sup>129</sup> reported that the brain in a fatal case did inactivate vaccine virus, giving a modicum of support to the explanation by autosterilization. Using cerebrospinal fluid for which normal vaccinated controls were available, Herzberg-Kremmer and Herzberg<sup>130</sup> found vaccine virus in one of three cases of postvaccinial encephalitis, although eighteen normal controls were negative. Gildemeister<sup>131</sup> also found no virus in the cerebrospinal fluid of fifteen normal vaccinated persons, but found it in the fluid of one of four cases of postvaccinial disease. Widowitz<sup>15</sup> found that the serums in two cases of postvaccinial encephalitis had

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121. McIntosh, J., and Blaxall, F. R. (footnote 3).

122. Aldershoff, H.: *Acta path. et microbiol. Scandinav.*, 1930, supp. 3, p. 9.

123. Bijl, J. P.: *Versl. med. Volksgezdh.*, 1927, p. 1471; quoted by Netter, *Presse med.* **37**:1469, 1929.

124. Fildes, P. (footnote 3).

125. Bastiaanse, F. S. van B.; Bijl, J. P., and Terburgh, J. T.: *Geneesk. tijdschr. v. nederl. Indie* **70**:1267, 1926.

126. Levaditi, C.; Lepine, P., and Troisier, J.: *Bull. Acad. de méd., Paris* **100**:818, 1928.

127. Maitland, C. (footnote 3).

128. Netter, A.: *Bull. Acad. de méd., Paris* **102**:255, 1929.

129. Bijl, J. P., and Fraenkel, H. S.: *Centralbl. f. Bakteriol. (Abt. 1)* **112**:412, 1929.

130. Herzberg-Kremmer, H., and Herzberg, Kurt: *Centralbl. f. Bakteriol. (Abt. 1)* **115**:271, 1930. Eckstein, A.; Herzberg-Kremmer, H., and Herzberg, Kurt: *Deutsche med. Wchnschr.* **56**:264, 1930.

131. Gildemeister, E.: *Deutsche med. Wchnschr.* **55**:1372, 1929. Gildemeister, E., and Hilgers, Paul: *Centralbl. f. Bakteriol. (Abt. 1)* **117**:322, 1930.

considerably less power of neutralizing the vaccine virus than did the serums of persons who were vaccinated about the same period of time before the withdrawal of serum, possibly indicating that the invasion of the brain is the result of an insufficient immunity reaction to the dermal infection. Efforts to detect the presence of other viruses in the brain in cases of postvaccinial encephalitis have been without result (herpes,<sup>132</sup> poliomyelitis<sup>133</sup>).

Are the lesions vaccinial? The similarity or dissimilarity of the lesions of brain and spinal cord in postvaccinial encephalitis and in experimental vaccinial encephalitis in animals is obviously of importance in determining the part played by vaccine virus in the etiology of the former. The essential pathology of the human disease has been briefly reviewed earlier in this paper. Levaditi and his co-workers<sup>134</sup> described the cerebral lesions resulting from the intracerebral inoculation of neurovaccine into rabbits and apes. They described the process as a meningo-encephalitis with involvement of the dura, pia mater and cortex. Perivascular and focal infiltration of these structures, first by polymorphonuclear cells and later by mononuclears, takes place. Condrea<sup>88</sup> gave a similar description, but also described Guarnieri's bodies as occurring in the nerve cells. Hurst and Fairbrother<sup>135</sup> regarded the essential lesion resulting from intracerebral inoculation of vaccine virus into rabbits and monkeys as a fibrinous, hemorrhagic and polymorphonuclear meningitis. They contrasted this picture with that in postvaccinial encephalitis, stressing in the latter the slight meningeal reaction, the strictly perivascular distribution of the lesions, the occurrence and the rapidity of demyelination, the microglial nature of the infiltration and the rarity of true perivascular infiltration by lymphocytes and plasma cells. They concluded that the pathologic pictures in the two diseases are so different as to make it extremely unlikely that vaccine virus is the cause of the postvaccinial condition.

On the other hand, in a number of studies McIntosh and his co-workers maintained that the lesions in postvaccinial encephalitis are essentially similar to those in vaccinial encephalitis in animals, to those in the various organs in generalized vaccinia in rabbits, to those in the brain in postvariolar encephalitis and to the focal lesions in smallpox.<sup>136</sup> In one case of postvaccinial encephalitis, McIntosh found a similar picture in the multiple lesions occurring in the internal organs. McIntosh

132. Maitland and Perdrau (footnote 3). Bijl, J. P., quoted by Gildemeister (footnote 44).

133. Levaditi, C., and Bijl, J., quoted by Gildermeister (footnote 44).

134. Levaditi and co-workers (footnotes 87 and 97).

135. Hurst, E. W., and Fairbrother, R. W.: *J. Path. & Bact.* **33**:463, 1930.

136. McIntosh, James; and Scarff, R. W.: *J. Path. & Bact.* **32**:551, 1929. McIntosh and Scarff (footnote 51). McIntosh (footnote 58).

and Scarff<sup>109</sup> did not regard the fibrinopurulent meningitis described by Hurst and Fairbrother as characteristic of vaccinia either in the skin or in the brain. With vaccine virus they produced in rabbits, two types of lesions, meningitis and meningo-encephalitis. They considered the difference between these as primarily one of severity. The presence of a diffuse radiating infiltration by a large number of cells with large, clear nuclei, giving the appearance of a tissue culture, was regarded as the characteristic feature of all the lesions mentioned. Demyelination was not regarded by McIntosh as of especial importance as a distinguishing factor, since it occurs in several unrelated pathologic conditions and has been observed by him in vaccinal encephalitis in the rabbit and monkey. Clearkin<sup>111</sup> found the picture described by McIntosh in the brains of wild monkeys inoculated intradermally with vaccine virus. He also found some degree of perivascular demyelination and regarded the lesions as essentially similar to the human postvaccinal lesions. Bijl and Fraenkel<sup>120</sup> were of the same opinion as McIntosh, that the histologic picture in the brain in postvaccinal encephalitis is essentially similar to that produced in the organs of rabbits by vaccine virus. Spooner<sup>137</sup> in a recent study found meningitis to be the most conspicuous lesion following injection of neurovaccine into the rabbit's brain. While certain changes occurred in the myelin sheaths, perivascular demyelination of the kind described in postvaccinal encephalitis was not seen. Agreeing with McIntosh as to the damage to, and proliferation of, the vascular endothelium Spooner regarded this feature as subsidiary to the general inflammation. The final decision regarding the vaccinal or nonvaccinal nature of the postvaccinal lesions awaits further reports confirming or denying McIntosh's descriptions.

**Serum Treatment:** Serum from vaccinated persons or animals has been used in treatment in a number of cases of postvaccinal encephalitis, with apparent benefit (Horder,<sup>47</sup> Rozendahl,<sup>119</sup> Hekman,<sup>138</sup> Netter<sup>139</sup>). The apparent beneficial action of this serum was regarded by Netter, among others, as a further demonstration of the vaccinal nature of the disease. However, controls treated with normal serum are not available, and statistical proof of the efficiency of the serum from vaccinated persons or animals is not yet possible, making its action rather unreliable evidence as to the etiology of the disease.

Some authors upholding the theory that vaccine virus is the direct cause have explained the increase in incidence of postvaccinal encephalitis in recent years by

137. Spooner, E. T. C.: *Am. J. Path.* **6**:767, 1930.

138. Hekman, J.: *Nederl. tijdschr. v. geneesk.* **73**:4774, 1930.

139. Netter, A.: *Tribuna med.* **64**:307, 1930; *Presse* ,

*Welt* **4**:

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assuming some change in the virus caused by its passage through rabbits, which is a comparatively recent procedure. Coplans, King and Simpson<sup>140</sup> emphasize the fact that encephalitis first occurred in England subsequent to the use of rabbit lymph. Netter<sup>130</sup> (1930) also connected the disease with the passage of the virus through rabbits. Other than the "post hoc" type, there seems to be no evidence in favor of this view. Neurovaccine passed through many generations of rabbits in Spain produced no encephalitis, although it did in Holland.<sup>141</sup> The only kind of lymph the use of which in Holland has not been followed by encephalitis is a rabbit strain from the Dutch East Indies.<sup>11</sup> A lymph from Japan and a bovine lymph from Switzerland, which had never been through rabbits, have been associated with encephalitis in Holland.<sup>141</sup>

Possibly the majority of the investigators concerned with the problem of postvaccinial encephalitis have been unable to accept the theory that vaccine virus is the cause for a number of reasons. Two of these have been discussed, involving the rarity or the lack of virus in the brain in fatal cases and the differences between the lesions in experimental encephalitis in animals and those in the postvaccinial disease. In the field of epidemiology there are a number of facts that are regarded as incompatible with this theory. The sudden and recent increase in incidence was cited by both the Andrewes and Rolleston committees as making it unlikely that vaccine virus is the only factor in the etiology. Many different strains of lymph have been associated with the complication, and it was considered extremely improbable that all these strains simultaneously acquired the property of producing changes in the central nervous system. The extremely irregular occurrence and the lack of direct proportionality of incidence to number of vaccinations also indicate the existence of some factor in addition to that of vaccine virus. The continued use, in Spain, of a strain of virus adapted to the brain of the rabbit (neurovaccine of Levaditi) for over two million vaccinations without the occurrence of a single case of encephalitis seems to indicate that the conditions in man and animal have no relationship, although it has been pointed out that when this strain was taken to Holland, a possibly greater proportion of cases resulted than when dermal strains were used.<sup>117</sup>

Some observers<sup>135</sup> have considered their inability to produce lesions in the brains of rabbits by cutaneous infection as evidence against the direct rôle of vaccine virus in the postvaccinial disease, but, as noted heretofore, others have occasionally been successful, and it must be pointed out that in human beings, in whom the cutaneous route of inoculation is always used, the incidence is extremely low (from 1 in 4,000 to 1 in 50,000).

140. Coplans, M.; King, W. G., and Simpson, W. J. R.: *Brit. M. J.* **2**:556, 1929.

141. Paschen, E.: *Deutsche med. Wchnschr.* **56**:219, 1930.

The epidemiologic features considered by many as making unlikely the hypothesis that vaccine virus is the cause do not seem to eliminate the possibility of direct action of vaccine virus in postvaccinal encephalitis. The supporters of this theory do not maintain that the inoculation with vaccine virus is the only factor in the disease. McIntosh, in his minority report to the Andrewes committee, stressed the presence of an "accessory factor," and Netter<sup>142</sup> mentioned the possibility of some peculiar "individual susceptibility" as explaining many of the epidemiologic peculiarities of the disease. Aycock,<sup>143</sup> in his studies on poliomyelitis, emphasized the fact that all who were exposed to the virus do not contract the disease, but that the majority are immunized without recognizable symptoms. He presented data that indicate that these different reactions to infection with the virus may be due to variations in the physiology of the hosts rather than to variations in the virus. The variations and the irregularity of the incidence of postvaccinal encephalitis might be explained on this basis. Zinnser<sup>144</sup> pointed out that the postvaccinal complication has occurred much more frequently in the northern than in the southern countries of Europe and has suggested that deficiency of vitamins might explain the differences in susceptibility.

#### ACTIVATION OF SOME OTHER VIRUS

The Andrewes Committee<sup>3</sup> referred to the activation of some concurrent infections, such as leprosy, by vaccinia and on this analogy based their suggestion of a possible activation of some virus, known or unknown, causing postvaccinal encephalitis. The fact that no known virus, other than that of vaccinia, has ever been isolated from the brain in fatal cases appears to indicate that the supposed activated virus is one at present unknown. Kraus and Takaki<sup>145</sup> claimed that by a special technic for the fixation of alexins they had demonstrated in one fatal case the presence of a virus related to herpes, but their work has not been confirmed. The most convincing evidence that the postvaccinal condition is caused by the activation of some unknown virus is found in the clinically and possibly pathologically similar conditions occurring independently and following other exanthems. These conditions have been described in some detail, and it has been emphasized that only in the cases following smallpox and measles and possibly some independent cases has a definite histologic similarity to postvac-

142. Netter, A., quoted by Paris correspondent, *J. A. M. A.* **93**:858, 1929.

143. Aycock, L. W.: *J. Prev. Med.* **3**:245, 1929.

144. Zinnser, H.: Carpenter Lecture, New York Academy of Medicine, 1930.

145. Kraus, R., and Takaki, J.: *Med. Klin.* **21**:1872, 1925. Kraus, R.: *Wien. klin. Wchnschr.* **40**:185, 1927.

cinial encephalitis been proved. McIntosh considered cerebral complications of smallpox as evidence of the vaccinal nature of postvaccinial encephalitis and regarded cases of encephalitis in measles as similar because caused by a virus having properties similar to the variola-vaccine viruses. The recent increase in the incidence of these similar conditions coincidental with the appearance of the vaccinal complication is stressed by the supporters of the activation theory,<sup>146</sup> who consider it difficult to assume a sudden rise in the encephalitogenic properties of all these viruses simultaneously. But it is likely that changes in individual susceptibility to one encephalitogenic virus would also make for greater susceptibility to other similar viruses, so that it is not necessary to assume a second virus to explain these coincidental variations in incidence. The Andrewes committee<sup>3</sup> considered the irregular grouping of postvaccinial cases as indicating the occurrence of small foci of carriers of a neurotropic virus activated by the vaccinations. Knöpfelmacher suggested that the supposed greater susceptibility of children of school age is due to the greater exposure of these children to possible encephalitogenic viruses. Greenfield<sup>60</sup> quoted a case reported by Lucas in which variolar encephalitis and encephalitis complicating measles occurred in the same patient some years apart and suggested this as evidence of activation of a latent virus; but possibly a more plausible explanation would be that the patient had a peculiar susceptibility to these encephalitogenic viruses. The cases occurring in the same families at long intervals could be explained on the same basis. On the experimental side Zurukzoglu<sup>147</sup> and Levaditi and Nicolau<sup>148</sup> attempted to show that vaccine virus is capable of activating subinfective doses of herpes virus, but Maitland and Gordon<sup>149</sup> were unable to confirm this.

The activation of, or the introduction of, bacteria or other larger forms by vaccination has been considered, but the great majority of workers have been unable to cultivate bacteria from the brain or cerebrospinal fluid in cases of postvaccinal encephalitis. Cases have been produced by the use of bacteriologically sterile neurovaccine, ruling out the introduction of bacteria by the lymph. Certain workers, however, have obtained results that suggest the activation of bacteria. Bijl (Gildemeister<sup>44</sup>) isolated from the brain in two cases a pleomorphic streptococcus that produced fatal encephalitis on intracerebral injection into rabbits. The lesions were not like the lesions in postvaccinial encephalitis, however. Aldershoff and Pondman<sup>150</sup> isolated *Bacillus bipolaris* from the lymph used and from the nasal discharges in a case. They proved that intracerebral vaccination in rabbits

146. Greenfield (footnotes 53 and 81).

147. Zurukzoglu, S.: *Klin. Wchnschr.* 6:70, 1927.

148. Levaditi, C., and Nicolau, S.; *Compt. rend. Soc. de biol.* 93:3, 1925.

149. Maitland and Gordon (footnote 3).

150. Aldershoff, H., and Pondman, A. B. F. A.: *Centralbl. f. Bakteriol.* (Abt. 1) 107:433, 1928.



activated *B. bipolaris* already present and were able to isolate the organism from the brains of these rabbits. Pondman and Pette (Gildemeister<sup>44</sup>) also showed an activation of *B. bipolaris* in vaccinated rabbits, but showed that it could produce only meningitis and not encephalitis. Kling, Lonberg and Wassen<sup>16</sup> found in the brain in fatal cases some round and oval bodies which they regarded as protozoa and considered were activated by the vaccination, and caused the cerebral lesions. Recently Aldershoff<sup>151</sup> reported finding certain yeast cells in the throats of persons with postvaccinial encephalitis and of contacts and in the cerebrospinal fluid of one, but never in the lymph used. Intravenous injections of the cultures of these organisms produced nervous symptoms in rabbits. A monkey nasally infected with the organisms showed nervous symptoms, and the yeast cells were found in the brain. The organism was found in the brain in six of eight postvaccinial cases and in poliomyelitic material which Aldershoff obtained from the Pasteur and Rockefeller Institutes. He considered this organism to be related to, if not identical with, the globoid bodies of Flexner, the streptococcus of Rosenow and the protozoon of Kling.

The assumption of a second unknown virus has been objected to by McIntosh on the ground that it introduces an unnecessary complication. It is, however, necessary to assume some factor in addition to vaccination to explain the peculiarities of the epidemiology, and the assumption of a virus might seem to be no more complicating than the assumption of a variation in susceptibility. But it is questionable whether the epidemiology can be explained by the assumption of an activated virus alone. By analogy with the virus of poliomyelitis a virus occurring in so many different areas would in time be generally distributed within these areas (especially the urban), and the irregular distribution of cases would still require a variation in susceptibility for its explanation. The lack of multiple cases in the same family, a feature of the postvaccinial disease, as well as of poliomyelitis, is much more easily explained by a difference in susceptibility than by the assumption that one child in the family is infected with the unknown virus, while the others are not.

*Allergy.*—A third possible etiologic explanation has been advanced by several observers. Starting with the fact that the period of incubation is the same as that required for the development of vaccinal immunity, Glanzman<sup>152</sup> suggested that the changes in the central nervous system result from a local anaphylactic reaction between the virus and the cellular antibodies. The virus in this process is simultaneously destroyed, which explains its absence from the brain. The theory in this form could be easily adapted to the theory that the vaccine virus is the cause if one agreed with Pirquet<sup>153</sup> that the essential process in infection with vaccine virus is an allergic one

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151. Aldershoff, H.: *Tribuna med.* **64**:307, 1930.

152. Glanzman, E.: *Schweiz. med. Wchnschr.* **57**:145, 1927.

153. Pirquet, C. E.: *Arch. Int. Med.* **7**:259, 1911.

and that the time necessary for the lesions to show themselves is the time required for the production of antibodies. Certain authors have postulated a less specific and more indefinite allergic process. Keller and Schaefer<sup>154</sup> considered the process one of sensitization to an already present virus. Rivers,<sup>33</sup> on the basis of certain nervous conditions produced in rabbits by the intradermal injection of streptococci and pneumococci and on the basis of the cases of paralysis produced by antirabic treatment, was inclined to give some measure of support to an explanation by allergy, but he did not enter into details as to the possible mechanism of such a process. Of interest in this connection is the case reported from China<sup>155</sup> in which urticaria followed a few minutes after vaccination and encephalitic symptoms occurred in ten days. While the assumption of such a basic allergic reaction to explain the various similar conditions discussed is attractive and with more evidence may prove to be correct, there is at present no evidence requiring the acceptance of such an explanation.

#### PROPHYLAXIS AND TREATMENT

Since the etiology of postvaccinial encephalitis is still in question, many measures for prevention or treatment must be tentative and of doubtful value. Various suggestions have been made by the committees appointed to study the problem and by independent authors. The following measures have been recommended: the use of only one insertion of lymph at vaccination; the dilution of the lymph used; primary vaccination in early infancy rather than later; the vaccination only of children in perfect health; rest in bed for the vaccinated one while the febrile reaction is present; cessation of vaccination in regions where epidemic nervous disease is present. Doerr suggested that subcutaneous injection of killed virus be tried in place of vaccination by living virus, but the problem of immunization by killed virus is still far from solution and this procedure will probably not be practicable until some means have been found for concentrating the virus. If the condition proves to be due to vaccinal infection of the brain, the fact, noted heretofore, that certain strains of virus produce cerebral lesions in rabbits readily, while other strains do not, may prove to be of importance. In the treatment of patients with postvaccinial encephalitis, it is probably advisable to administer serum from some person recently vaccinated, although proof of the efficacy of this is still lacking.

It is important to emphasize the extremely low incidence of postvaccinial encephalitis, the incidence being especially low in this country. There is no necessity for any cessation of vaccination, since the benefits gained enormously outweigh the possibilities of danger; but cases that occur should be reported, so that all the information possible can be obtained.

#### SUMMARY

The three most important etiologic theories may be briefly considered again: There is much evidence in favor of the assumption that post-

154. Keller, W., and Schaefer, W.: *Jahrb. f. Kinderh.* **125**:253, 1929.

155. McClure, W. B.: *China M. J.* **44**:526, 1930.

vaccinial encephalitis is caused by the direct action of vaccine virus on the brain, but two important objections to this are not yet satisfactorily explained—the lack of vaccine virus in the brain in most cases and the differences between the lesions in man and those produced experimentally in animals. The work of McIntosh may, with further corroboration, remove the second obstacle. The epidemiologic facts apparently opposed to this theory can be explained by the assumption of variations in susceptibility to the encephalitogenic action of the vaccine virus. The theory of an activation of some unknown virus is largely supported as an alternative explanation by those who regard the obstacles to the acceptance of the first explanation as unsurmountable. There is little, if any, positive evidence in favor of this theory which cannot be equally well explained on the basis of the theory that the vaccine virus is the cause. The conception of an allergic reaction as an explanation has no direct evidence in its favor.

## Notes and News

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**University News, Appointments, Promotions, Resignations, Deaths, etc.**—In the Rockefeller Institute for Medical Research, Oskar Seifried has been promoted from associate to associate member, and George P. Berry, Elmer E. Fleck, Thomas Francis, Jr., Raymond C. Parker and Robert E. Steiger from assistants to associates.

In the school of medicine of the Creighton University, Omaha, Thomas McCurdy and Clarence Moran have been appointed assistants in pathology; M. W. Barry has resigned as instructor in pathology.

Max Cutler has resigned as director of research and attending radiation therapist in the New York City Cancer Institute to accept the directorship of the tumor clinic of the Michael Reese Hospital in Chicago.

Taichi Kitashimi has been elected director of the Kitasato Institute for Infectious Diseases, Tokio, to succeed the late Shibasaburo Kitasato.

New appointments to the staff of the New York Hospital-Cornell Medical College Association include Eugene L. Opie, now professor in the University of Pennsylvania, as professor of pathology and pathologist to the hospital, and James M. Neill, now professor in Vanderbilt University, as professor of bacteriology and immunology. James Ewing retires from the professorship of pathology at Cornell, which he has filled since 1899, and will devote himself to the study of cancer and to the Memorial Hospital where he is president of the medical board.

**Departmental Bibliography in Place of Collected Reprints.**—Instead of supplying bound volumes of reprints, as has been the custom, the department of pathology of Columbia University, New York, has prepared a list of the recent publications by its members, reprints of which may be obtained on request.

**Recommendations About Postmortem Examinations in New York City.**—Recommendations to bring about cooperation between hospital authorities and funeral directors in the matter of postmortem examinations are set forth in the report of a joint committee representing the New York Academy of Medicine, the New York Pathological Society and the Metropolitan Funeral Directors' Association. The committee urges that hospitals avoid unnecessary delays in obtaining permission for necropsies, that they should obtain necessary data for a death certificate when a patient is admitted and that they arrange to inform funeral directors promptly when bodies are ready. The funeral director on his part must recognize the obligation of the hospital to obtain permission from the family of the deceased for examination. He must present to the hospital acceptable written authority from the family to take charge of a body. Other recommendations concern the selection of funeral directors by hospital authorities, reports of death to the medical examiner and the technic of necropsy. It is suggested that an embalmer be present at the examination. Finally, the committee recommends the appointment of a continuing joint committee representing the same organizations with the addition of a representative of the hospital executives. The report was approved by the council of the New York Academy of Medicine.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

STUDIES ON THE "ACID DEFICIT" IN PERNICIOUS ANEMIA. JOSEPH E. CONNERY and NORMAN JOLLIFFE, *Am. J. M. Sc.* **181**:830, 1931.

A return of free acid in the gastric contents is reported in one patient suffering from pernicious anemia. In eleven cases of pernicious anemia no change in "acid deficit" could be demonstrated following liver treatment over a period of from one to fourteen months.

AUTHORS' SUMMARY.

THE RELATION OF BROCA'S CENTER TO LEFTHANDEDNESS. KARL ROTHSCHILD, *Am. J. M. Sc.* **182**:116, 1931.

Location of speech center and preference for one side of the body stand in only loose connection. Location of Broca's center in the right hemisphere is compatible with right handedness. Description of two such cases, discovered following apoplectic insults, is herewith presented. In both cases there are left handed and right handed persons in the family. A combination of both "seitigkeitsanlagen" produces true left sidedness.

AUTHOR'S SUMMARY.

FURTHER STUDIES IN A CASE OF CALCIFICATION OF SUBCUTANEOUS TISSUE ("CALCINOSIS UNIVERSALIS") IN A CHILD. W. BAUER, A. MARBLE and G. A. BENNETT, *Am. J. M. Sc.* **182**:237, 1931.

Further studies in a case of "calcinosis universalis" are reported. Studies of calcium and phosphorus balance indicated a marked tendency to retain the absorbed calcium and phosphorus in spite of normal blood calcium and phosphorus values. This tendency was more marked in the case of calcium than phosphorus. Ammonium chloride acidosis produced an increase in the urinary excretion of calcium and phosphorus. Chemical analysis of a calcareous nodule removed at biopsy showed the presence of a negligible quantity of uric acid, a moderate amount of cholesterol and fatty acids, and calcium and phosphorus in the amount and ratio, one to the other, commonly found in adult bone and pathologic human calcification. Histologic study showed no evidence of antecedent tissue necrosis, nor did it suggest any explanation for the abnormal calcium deposits. The foregoing results suggest that the basis of the disorder lies in an abnormality of calcium and phosphorus metabolism. It is suggested that the increased retention of calcium and phosphorus may be the result of local cellular conditions as yet undetermined.

AUTHORS' SUMMARY.

THE EPITHELIOID CELLS. R. S. CUNNINGHAM and EDNA H. TOMPKINS, *Am. Rev. Tuberc.* **23**:71, 1931.

It seems that many substances introduced into the subcutaneous connective tissues are capable not only of producing accumulations of large mononuclear cells, but of modifying the morphology and physiology of these cells in different ways. When the material introduced into the tissues interferes with the normal metabolism of the cell it is probable that epithelioid cells are produced. This seems true whether the interference acts from within or without the cells. The authors wish to present their ideas merely as a different approach to cellular physiology and pathology than has previously been possible.

H. J. CORPER.

NUTRITIONAL MUSCULAR DYSTROPHY. M. GOETTSCH and A. M. PAPPENHEIMER, *J. Exper. Med.* **54**:145, 1931.

A diet is described that leads to a progressive, highly selective, and ultimately fatal dystrophy of the voluntary muscles. Guinea-pigs and rabbits are susceptible, rats resistant. The diet used is complete in known requirements, except for vitamin E; the addition of this factor, however, does not prevent the development of the disease. The lesions are not due to inanition, infection or scurvy, and must be referred to some still unknown factor.

AUTHORS' SUMMARY.

NERVE ENDINGS IN MUSCULAR DYSTROPHY. W. M. ROGERS, A. M. PAPPENHEIMER and M. GOETTSCH, *J. Exper. Med.* **54**:167, 1931.

The nutritional muscle dystrophy of guinea-pigs and rabbits is primarily a degeneration of the muscle fibers and is not associated with visible alterations of the peripheral nerves or their motor terminals.

AUTHORS' SUMMARY.

EFFECT OF TESTICLE EXTRACT ON RED CELLS. G. FAVILLI, *J. Exper. Med.* **54**:197, 1931.

Testicle extract possesses a notable power to increase red cell fragility. Testicle extracts of the rat, rabbit and guinea-pig all exhibit this property, which is most pronounced with the extract of the rat and least so with that from the guinea-pig. Splenic extract does not possess the property, or possesses it only to a very slight degree. These results support the hypothesis that the factor in testicle extract that enhances infections (the Reynals factor) does so by altering the permeability of the host tissue.

AUTHOR'S SUMMARY.

PYOCYANINE, AN ACCESSORY RESPIRATORY ENZYME. E. A. H. FRIEDHEIM, *J. Exper. Med.* **54**:207, 1931.

Pyocyanine, the blue pigment of *B. pyocyaneus*, can increase the respiration of living cells to a great degree (maximum observed increase twenty-four fold). The reversibility of its oxidation and reduction is responsible for this. The effect is nonspecies-specific and has been observed in varying degrees with *B. pyocyaneus*, *Staphylococcus aureus*, *Pneumococcus* type III and the red blood corpuscles of rabbits. The effect of pyocyanine is dependent on the presence of another respiratory ferment sensitive to potassium cyanide and carbon monoxide. The increase of respiration induced by pyocyanine is paralleled by an increase in the respiratory quotient. The pyocyanine catalysis is not indiscriminately effective in all oxidations, but only in the oxidation of certain substances closely associated with the bacterial body.

AUTHOR'S SUMMARY.

AGE OF LYMPHOCYTES IN PERIPHERAL BLOOD. B. K. WISEMAN, *J. Exper. Med.* **54**:271, 1931.

The study of blood from rabbits with normal and with hyperactive lymphatic tissue reveals, in the latter, a greater percentage of lymphocytes with heavily basophilic cytoplasm and numerous mitochondria. This indicates that cytoplasmic basophilia and mitochondrial content can serve as criteria of the degree of maturity of the lymphocyte, these characters having the same significance in this relation as obtains with other blood cells. Basophilia is the more evident and reliable indicator of youth of the cells. The classification of lymphocytes into three groups, according to degree of basophilia, has yielded figures that show the proportions of the three to be relatively stable in blood from normal adult human beings and rabbits. Size is not strictly a function of age in lymphocytes. Moreover, there is no correspondence in the size of lymphocytes in supravital films and in fixed

specimens obtained by the "cover glass" method. There is a change of size during fixation. Although lymphocytes of intermediate and large size may be of any age, in supravital preparations the majority are young cells, whereas in fixed films the reverse obtains. The small lymphocyte may be of any age in specimens examined by either technic. The total number of lymphocytes circulating at any given time is not necessarily an index to lymphoid activity.

AUTHOR'S SUMMARY.

MYOCARDIUM IN YELLOW FEVER. WRAY LLOYD, University of Toronto Studies, Pathologic Series, No. 8: University of Toronto Press, 1931.

An attempt at a physiopathologic correlation has been made in a myocardial disease that can be reproduced alone in a healthy animal and made the basis of a laboratory experiment. Attacking the problem in this fashion, an especial effort has been extended to answer two questions proposed for solution, when the problems of the study were first stated. At this time, the need of determining the cause of the bradycardia in yellow fever and of learning the significance and constancy of the degenerative lesions of the myocardium was stressed. A solution to the former is strongly suggested in the degenerative lesion of the sino-atrial node, and an answer to the latter is found in the parallelism of occurrence of the electrocardiographic evidences of altered function with the appearance of degenerations in the auricular muscle, auriculoventricular bundle and ventricle.

AUTHOR'S SUMMARY.

EFFECTS OF HEMORRHAGE ON THE VASCULAR NERVOUS MECHANISM. A. CHAUCHARD, B. CHAUCHARD and D. T. BARRY, Brit. J. Exper. Path. **12**: 190, 1931.

Modifications of the excitability of the parasympathetic inhibitory mechanism of the heart and of the vasomotor mechanism are shown to occur as a result of hemorrhage in dogs. Changes of the rheobasis are not distinctive in value, and this holds for both peripheral and central stimulation of the inhibitory apparatus and for central stimulation of the vasomotor. The chronaxia, on the other hand, is invariably increased by loss of blood for all the reactions tested, and the extent of the increase is roughly proportional to the severity of the hemorrhage. Restoration of the blood lost, after defibrination, causes more or less complete return to former values of excitability. Temporary recovery of these values is also determined by injection of saline solution.

AUTHORS' SUMMARY.

THE CAUSES OF ICTERUS NEONATORUM. K. J. ANSELMINO and F. HOFFMANN, Arch. f. Gynäk. **143**:477, 1931.

The cause of the increased amount of hemoglobin, erythrocytes, glutathione, total blood volume and blood catalase, relative increase in the weight of the heart and rapid pulse in the fetus lies in the condition of lowered oxygen tension under which it lives. The sum of the alterations mentioned is entirely similar to the changes noted both in mountain climbers and in animals kept experimentally under conditions of lowered oxygen tension identical with those of high altitudes. The oxygen tension in the maternal uterine artery averages 14.56 per cent by volume, whereas the oxygen tension in the corresponding fetal umbilical arteries is only 3.53 per cent by volume—about one-fourth that of the maternal blood. The average hemoglobin is 24 Gm. per hundred cubic centimeters (150 per cent) and erythrocyte count 6,500,000 per cubic millimeter. The total blood volume averages 12 per cent of the body weight, an increase of 50 per cent above normal. In the acclimatization of the new-born infant to the conditions of atmospheric air the hemoglobin rapidly undergoes decomposition and bilirubin in the blood plasma rapidly rises for several days after birth, just as it does in animals experimentally acclimatized to conditions of high altitude and suddenly reacclimatized

to oxygen tension at normal altitude. This unusually high bilirubin content of the plasma offers a part of the explanation of the occurrence of icterus. However, it fails to explain why icterus does not occur in about 20 per cent of the new-born infants.

A second factor, that of variability in the permeability of the cutaneous capillaries, is very important. Localized icterus can be produced in nearly all new-born infants by the cataphoretic introduction of histamine into the skin. The increased permeability of the capillaries so produced causes the visible appearance of icterus in the area of skin treated.

LAWRENCE PARSONS.

ON THE ORIGIN OF HYPOPHYSEAL CACHEXIA (SIMMOND'S DISEASE). W. MERZ, Frankfurt. *Ztschr. f. Path.* 40:452, 1930.

Two cases are reported. The first case was that of a 35 year old man who showed progressive loss of weight, gastric distress, alimentary glycosuria, decreased basal metabolism, increasing weakness and neurasthenia. These symptoms were manifest for seventeen years and were first noticed shortly after an attack of angina. At autopsy, a colloid cyst of the size of a pea was found in the midportion of the hypophysis. The cyst had led to a diminution of the size of the anterior lobe and, to a less degree, of the posterior lobe. Histologically, there was a decrease of all the elements of the anterior lobe, but especially in the number of eosinophilic cells. In the region of the infundibulum, a marked sclerosis and atrophy was found. The thyroid revealed atrophic changes. The diagnosis of hypophyseal cachexia was made even though a few typical symptoms, such as atrophy of the genitalia and loss of hair, were not present. The author suggests that a toxin following angina rather than the colloid cyst had led to the changes of the anterior lobe of the hypophysis and to the hypophyseal cachexia. The second case was that of a 44 year old woman. Her first clinical symptoms were characteristic of exophthalmic goiter. Both superior thyroid arteries were ligated. Two months after the operation, she started to lose weight and developed a cachexia which became progressively worse. There were no changes in the carbohydrate metabolism. The autopsy revealed a marked atrophy of the hypophysis which weighed 0.4 Gm., pseudocirrhosis and atrophy of the liver, cortical contraction of the suprarenals, atrophy of the ovaries and mammary glands and a colloid goiter with hyperplasia. The crines pubis were markedly reduced. The anterior lobe of the hypophysis histologically showed a marked sclerosis and actual scar tissue. In several places, foci of lymphocytic infiltrations were found. The eosinophilic cells were reduced in number. The author's opinion is that a cytotoxin appeared following exophthalmic goiter, which produced the changes in the hypophysis, suprarenals, ovaries and liver. The case presents a combination of exophthalmic goiter and hypophyseal cachexia which, as the author states, is not known in the literature.

O. SAPHIR.

GENETICS AND PATHOLOGY. S. G. LEWIT, Frankfurt. *Ztschr. f. Path.* 40:552, 1930.

This article deals with the etiology and differentiation of diseases biologically. Much weight is put on heredity. The individual variation of the same disease is discussed from various angles. An attempt is made to explain a number of diseases by a certain constitution (genotype) which is not apparent until external influences make this constitution manifest in the form of a disease. Tuberculosis is an example of this type. Another group of diseases is caused entirely by the genotype of the individual and does not need external influences to become manifest. Hemophilia is an example of such a disease. The third group embraces diseases that are produced primarily by external causes, as are most infectious diseases. This type of disease is referred to as paratyphic.

O. SAPHIR.



THE INFLUENCE OF THE FEMALE SEX HORMONE AND THE ANTERIOR HYPOPHYSEAL HORMONE ON THE STRUCTURE OF THE HYPOPHYSIS. B. ZONDEK and W. BERBLINGER, *Klin. Wchnschr.* **10**:1061, 1931.

The morphologic changes caused in the anterior lobe of the hypophysis of the rat and mouse by castration are not modified by the prolonged use of the female sex hormone. A single dose of the follicle-ripening hormone of the anterior lobe of the hypophysis stimulates certain maturation changes in that lobe in the young animal. Repeated doses of the follicle-ripening and luteinization hormone cause a definite maturation of the anterior lobe.

AUTHORS' SUMMARY.

PLETHORA. L. ASCHOFF, *Verhandl. d. deutsch. path. Gesellsch.* **25**:106, 1930.

Volumetric studies of the blood from the heart and thoracic aorta in a hundred autopsies disclosed an increased volume in the following conditions in order of importance: essential hypertension with hypertrophic heart and without arteriosclerosis, arteriosclerosis, obliteration of the pleural cavities, primary contracted kidneys with hypertrophic heart, right-sided cardiac hypertrophy and its related causes, toxic goiter and frank valvular lesions. The smallest volume were found in acute effusions into the abdominal cavities, fatal peritonitis, sepsis with marked perspiration and evaporation, cachexia of carcinoma, and hypertension with secondary contracted kidneys. Blood volume increases more rapidly than heart weight, in the ratio of 2:3.5. Edema, state of coagulation or its lack, regional distribution has no direct effect on blood volume. The increase in blood volume might be interpreted as an expression of disturbed circulatory mechanism possibly due to chemical disturbances. In heart failure from varying causes it might be explained as a compensatory process to fill vessels otherwise not distended. The increase of blood volume in essential hypertension is not explained.

SOL ROY ROSENTHAL.

THE GENERALIZED CHANGES IN INFLAMMATION. B. FISCHER-WASELS, *Verhandl. d. deutsch. path. Gesellsch.* **25**:115, 1930.

The injection of kieselguhr subcutaneously into mice and rabbits called forth a granulomatous and a necrotic reaction with suppuration. The generalized reaction resulting from reabsorption of protein-split products was as follows: anatomically, by a rounding of the reticulo-endothelial cells of the liver and spleen with swelling of the nuclei and basophilic staining of the cytoplasm; functionally, by their mobilization as seen by an increased absorption of trypan blue and casein, and metabolically, by the increased oxygen consumption. In granulomatous lesions, the oxygen consumption is slight as compared to the necrotic suppurative lesions. After evacuation of the pus in the necrotic lesions the oxygen metabolism returns to normal.

SOL ROY ROSENTHAL.

ORGAN METABOLISM BY TISSUE ACTIVITY AND ANAPHYLAXIS. W. BÜNGELER, *Verhandl. d. deutsch. path. Gesellsch.* **25**:125, 1930.

Intravenous injections of casein and horse serum into white rats and subsequent determinations of oxygen metabolism of liver, spleen, kidneys and lungs by Warburg's gasometric methods gave the following results: (a) In animals killed immediately after injection, small injections increase and large injections decrease the oxygen metabolism during the early stages (first five hours); later there is a constant rise which lasts twenty-four hours and is followed by return to normal. (b) During the early stages of phagocytosis of acid dyes, increased oxygen consumption was found in the liver, kidneys and other organs, but after phagocytosis is completed organ metabolism is again normal. (c) Intravenous injection of india ink and immediate killing of the animal with subsequent deter-

mination of metabolism showed that only when phagocytosis had ceased did oxygen metabolism return to normal, and then only slowly. (d) Guinea-pigs, white mice and rabbits killed during typical anaphylactic shock showed marked decrease of oxygen metabolism. In animals not sufficiently prepared or sufficiently prepared but not sensitized, there was marked increase in oxygen metabolism, especially in the liver and spleen.

SOL ROY ROSENTHAL.

PHAGOCYTOSIS OF COLLOID BODIES IN LIVING BODY AND IN TISSUE CULTURES. J. TANNENBERG, *Verhandl. d. deutsch. path. Gesellsch.* **25**:128, 1930.

The author seeks to prove that phagocytosis is not wholly dependent on the increase of particles brought to the cell by the circulation or by the surface area of the cells. That there is an increase of phagocytosis by a slowing of the circulation, the author shows by his injections of india ink, but the injection of trypan blue (which is held in fine colloid suspension), macrophages, histiocytes and fibroblasts all having equal exposure to the dye results in a greater phagocytosis by the macrophages than by either fibroblasts or histiocytes. This he interprets as a difference in function, dependent on the structure of the cell, which much be considered as a potent factor in phagocytosis.

SOL ROY ROSENTHAL.

THE PATHOGENESIS OF IRRADIATED CHOLESTEROL POISONING. G. SCHRETTTER and L. HASLHOFER, *Ztschr. f. d. ges. exper. Med.* **76**:352, 1931.

When large doses of irradiated cholesterol were given to dogs over a long enough period of time, the nitrogenous residuum in the blood became markedly increased. No lesions were found in the glomerular tufts of the kidneys. The suprarenals, liver and brain showed no changes sufficient to produce the high blood nitrogen. Therefore, it is suggested that the disturbance may be metabolic in origin.

PEARL ZEEK.

THE RÔLE OF THE SPLEEN IN METABOLISM. S. G. STSCHEDROWITZKY and S. A. SELTZER, *Ztschr. f. d. ges. exper. Med.* **76**:369, 1931.

Splenectomy in dogs was followed by a slight increase in thyroid function, increased alkali reserve in the blood, decreased blood sugar, increased blood cholesterol, increased blood calcium and lymphocytosis. Both normal and splenectomized dogs showed a decrease in blood calcium following the ingestion of spleen. Similar feeding caused no change in blood sugar or cholesterol in normal animals, but in splenectomized dogs the blood sugar was raised and the cholesterol lowered. It is suggested that the spleen may produce a hormone and may be closely related to glands of internal secretion.

PEARL ZEEK.

EXPERIMENTAL LEUKOSIS IN THE FOWL. O. THOMSEN and J. ENGELBERTH-HOLM, *Acta path. et microbiol. Scandinav.* **8**:121, 1931.

The paper describes and discusses the results obtained in an effort to produce leukosis in the fowl by injections of tar emulsion into the bone marrow. A form of tar was used that had been found to produce carcinoma of the skin in mice. Of sixty-two chickens in which tar was injected into the bone marrow every fifth day, nine developed extensive myeloid hyperplasia in various organs and tissues, which resembled closely the changes observed in spontaneous myeloid leukosis in the fowl. In some of the animals there was an increase in the leukocytes in the blood. Repeated intravenous injections of tar emulsion over a period of several months have not had any effect.

## Pathologic Anatomy

ANOMALY OF THE BILIARY TRACT. ANTONIO GENTILE, *Am. J. M. Sc.* **182:95**, 1931.

A case is reported of communicating ducts between the hepatic and cystic ducts, separate entrances of the common and hepatic ducts into the duodenum and an occlusion of the common duct. It is presented as a probably unique congenital anomaly of the biliary tract. An attempt is made to explain the embryologic mechanism underlying this anomaly.

AUTHOR'S SUMMARY.

INTRAHEPATIC LITHIASIS. HARRY KOSTER and I. E. GERBER, *Am. J. M. Sc.* **182:99**, 1931.

A case of intrahepatic lithiasis is presented with unusually large stones. A striking feature of this case is the freedom from symptoms during the long period in which the development of the stone undoubtedly occurred. A discussion of the causes and associated features of the development of stone is presented.

AUTHORS' SUMMARY.

A PERSISTENT OSTIUM ATRIOVENTRICULARE COMMUNE WITH SEPTAL DEFECTS IN A MONGOLIAN IDIOT. G. M. ROBSON, *Am. J. Path.* **7:229**, 1931.

A case is reported in which a persistent ostium atrioventriculare commune is associated with a defect in the base of the interventricular septum and a persistent ostium primum. This occurred in the heart of a Mongolian idiot who showed also a complete absence of true ovarian tissue. The cardiac defect is believed to be due to faulty development of the endocardial cushions. Four of the nine similar cases found reported in the literature occurred in Mongolian idiots.

AUTHOR'S SUMMARY.

THE SPECIFIC CHARACTER OF TOXIC CIRRHOSIS IN CINCHOPHEN POISONING. D. C. BEAVER and H. E. ROBERTSON, *Am. J. Path.* **7:237**, 1931.

Preparations of cinchophen have been shown definitely to be toxic for certain persons. The toxic effects are directed most severely and specifically against the liver. Unknown factors, apparently independent of the quantity of the drug used, appear to be significant in creating a predisposition or idiosyncrasy for the drug. Various grades and stages of hepatic degeneration have been described. These are presumably dependent on the completeness and rapidity of the initial reaction. The reaction may be rapid and complete, with the induction of acute atrophy of the liver, or slower and less complete, with apparent recovery. Intermediate between these two extremes subacute forms of intoxication may ensue and may become manifest both clinically and pathologically as a type of hepatic atrophy or cirrhosis, which corresponds in its anatomic characteristics to the distinctive toxic cirrhosis as described by Mallory. The clinical and anatomic characteristics of toxic cirrhosis appear to be specific and essentially dissimilar to the ordinary Laënnec or portal type. The clinical data and correlated studies of pathologic anatomy in five cases of intoxication from cinchophen constitute the basis for this study.

AUTHORS' SUMMARY.

GRANULOMATOUS ABSCESS OF THE LIVER OF PYOGENIC ORIGIN. D. C. BEAVER, *Am. J. Path.* **7:259**, 1931.

Abscesses of the liver, originating from within the field of drainage of the portal vein, form a significant clinical and pathologic group. They usually take origin from primary intestinal foci through the production of local thrombo-

phlebitis. The hepatic suppuration develops from the passage of infected emboli from the primary foci by way of the portal circulation to the liver. The primary thrombophlebitis may induce thrombosis of the portal vein and pylephlebitis, or this feature of the lesion may be absent. As the cases emphasize, the primary focus may be cryptogenic. From the appearance of the abscess, however, the source may be suspected. A characteristic distribution and type of abscess are produced by each method of hepatic dissemination. The abscesses of portal origin are at first multilocular, owing to the multiple foci in emboli. Each multilocular abscess usually remains discrete, although it may be multiple. Involvement of the right lobe alone is most common. General hepatic dissemination, in this type, is unusual. The significance of the pyogenic cocci as etiologic agents has been emphasized. The illness associated with abscess of the liver may be extremely insidious, and as the primary focus may be cryptogenic, so also the hepatic lesion itself may exhibit this same characteristic. Progression into extreme chronicity may occur, with preservation of the original multilocular arrangement, or a solitary, adequately encapsulated abscess may result. In the cases of extreme chronicity a granulomatous reaction has been found, with the pyogenic cocci demonstrable as the etiologic agents. Such cases resemble the granulomas of actinomycosis in their chronicity and in their granulomatous characteristics. Eight cases representative of pyogenic abscesses, possessing granulomatous changes in various stages of their evolution, have been presented.

AUTHOR'S SUMMARY.

ERYTHROBLASTOSIS WITH JAUNDICE AND EDEMA IN THE NEWLY BORN. J. A. FERGUSON, *Am. J. Path.* 7:277, 1931.

In the six cases reported, in all of which the patients died during birth or shortly after, a detailed description of the pathologic changes has been given. The outstanding feature was the occurrence of abnormal extramedullary hematopoietic activity in full term nonsyphilitic infants. In each instance there was a persistence of the fetal mode and location of blood-forming activity without a corresponding retardation in general embryologic development. Three cases showed marked jaundice at birth. In one of these, bile stasis was present in the liver. This may have been due to excessive hemolysis of large numbers of imperfectly differentiated erythrocytes loading the liver cells with bile pigment more rapidly than it could be taken care of by the bile-excreting apparatus. One of the three cases showing jaundice was complicated by an infection, but this was not thought to be related to abnormal hematopoietic activity or to the jaundice. Two cases showed marked edema at birth, which corresponded to the condition known as hydrops congenitus. One case showed neither jaundice nor edema. All cases showed a marked enlargement of the spleen and liver. The features which these cases have in common with well known disease conditions of infancy have been discussed. A few factors that are thought to be of etiologic significance have been mentioned. However, the cause is still unknown. The cases described in this report, when considered as a group, are probably representative of a definite disease entity of the newly born, and whatever the etiology may be, the underlying cause is undoubtedly the same in each instance, whether or not the individual case is characterized by jaundice or edema, or whether both jaundice and edema are lacking. The term erythroblastosis in the newly born is applied to the pathologic syndrome described in the six cases reported here because it best depicts the anatomic changes.

AUTHOR'S SUMMARY. }

STREPTOCOCCUS HEPATITIS. H. E. MACMAHON and F. B. MALLORY, *Am. J. Path.* 7:299, 1931.

The more common inflammatory changes in the liver in cases of streptococcus infection with and without septicemia are described. Emphasis is laid on a less common lesion, of which three cases are given in detail. This is characterized by

focal or diffuse areas of liver tissue showing necrobiotic changes and necrosis, infiltrated with an inflammatory exudate. A Gram-Weigert stain shows streptococci in large numbers in the lesions of two of these livers. The similarity of this lesion to the histologic picture at times encountered in acute yellow atrophy is discussed, and the suggestion is made that a careful bacteriologic search of the liver in the fixed preparation together with a culture of the liver at the time of the autopsy might reveal bacteria within the lesions more commonly than is suspected—particularly in those cases of so-called acute yellow atrophy showing a very irregular distribution of the lesion—a condition that is extremely difficult to explain purely on the basis of a circulating toxin in the blood. Another case is described with a chronic inflammatory reaction within the liver, showing on the one hand degeneration, necrosis, exudation and bacteria, and on the other a very active proliferation of bile ducts and connective tissue. This case is presented more for discussion than as a proved case of chronic progressive cirrhosis of infectious origin. The last point that is considered is the histologic and gross changes that one may find in the healed stage of these acute and chronic inflammatory lesions. The second part of the paper is devoted to the results of experimental work. A streptococcus obtained from an early case of scarlet fever was injected free from toxin into one of the radicals of the portal veins of both guinea-pigs and rabbits. The animals were killed at varying intervals, and the lesions produced, together with the results of bacteriologic studies, are fully described and compared with the lesions seen in cases in human beings.

AUTHORS' SUMMARY.

PULMONARY ASBESTOSIS. WILLARD B. SOPER, *Am. Rev. Tuberc.* **22**:571, 1930.

A case of typical asbestosis is reported. The most common single symptom of pulmonary asbestosis is dyspnea. This and the other symptoms are essentially those of a generalized progressive fibrosis of the lungs. The physical signs are substantially those of generalized fibrosis of both lungs and basal pleurisy. Asbestos contains but a small amount of free silica, but probably conduces to a more hasty evolution of any accompanying tuberculosis as in the better understood forms of silicosis. An immediate diagnosis at autopsy is made possible by simply squeezing out a drop of juice from the lung when the asbestosis bodies in large numbers are readily visible under the microscope.

H. J. CORPER.

ENCEPHALITIC, IDIOPATHIC AND ARTERIOSCLEROTIC PARKINSONISM: A CLINICOPATHOLOGIC STUDY. MOSES KESCHNER and PAUL SLOANE, *Arch. Neurol. & Psychiat.* **25**:1011, 1931.

Anatomic observations are contrasted in seven cases of parkinsonism that were observed clinically for about seven years. Three of the cases were diagnosed as chronic encephalitic parkinsonism, two as idiopathic or genuine Parkinson's disease and two as arteriosclerotic parkinsonism. The changes in the three types were most frequent and marked in the substantia nigra and globus pallidus (the pallidonigral system); the locus caeruleus was practically as frequently involved as the substantia nigra. The pallidum seemed to have been affected mostly in the idiopathic cases. The cerebellum was intact, while other basal formations (the putamen, optic thalamus, red nucleus and hypothalamus) were involved in the three types. The changes in the encephalitic type were both degenerative and inflammatory; the latter, however, may be quite mild, when the case may resemble the genuine type of parkinsonism. In these cases inflammatory perivascular infiltrations were absent. The arteriosclerotic form could be suspected from the condition of the arteries. The anatomic changes are not characteristic enough to permit of a differential diagnosis between the three types. In all of them the process is diffuse and is hardly ever localized. For this reason it is not possible to correlate the anatomic observations with the clinical picture.

GEORGE B. HASSIN.

THE ORIGIN AND FORMATION OF SENILE PLAQUES. ARMANDO FERRARO, Arch. Neurol. & Psychiat. **25**:1042, 1931.

Ferraro found that the senile plaques originate from nerve cells, oligodendroglia (the glia nuclei of Nissl) and especially microglia cells. In rare instances fat granule bodies are found in them. The oligodendroglia and microglia undergo disintegration, with the formation of a granular amorphous substance. This constitutes the argyrophile substance of the plaque, thus contributing to the increase in size of the plaques. Surrounding the plaques, usually at some distance, are numerous astrocytes, mainly as a reaction. Sometimes they form a part of the plaque, when they degenerate and thus also contribute to the increase in size of the plaque. The axis cylinders of a plaque are broken up. Ferraro believes that oligodendroglia and microglia cells may undergo some particular changes and constitute a small plaque.

More numerous are the plaques developing from microglia cells. Minute plaques may also originate from transformed nerve cells. From the various single elements forming small plaques, larger plaques form either by fusion of the former or by degeneration of oligodendroglia and microglia. Nerve fibers never give origin to senile plaques, but contribute to their growth through a process of fragmentation and gradual disintegration of the neurofibrils.

GEORGE B. HASSIN.

ENCEPHALITIS PERIAXIALIS DIFFUSA (SCHILDER'S DISEASE). C. DAVISON and W. SCHICK, Arch. Neurol. & Psychiat. **25**:1063, 1931.

A detailed clinical and pathologic report of Schilder's disease is given because of the unusual features: marked signs of extrapyramidal disorder and bilateral optic atrophy. The striking pathologic features were the bilaterality of the process, which extended from the frontal to the occipital lobe, and involvement of the corpus callosum, optic nerves, ependyma and basal ganglions. The changes were mainly degenerative, probably due to a toxin. The authors offer to call Schilder's disease encephalopathia periaxialis diffusa.

GEORGE B. HASSIN.

THE NERVOUS LESIONS OF SUBACUTE OR CHRONIC EXPERIMENTAL POLIOMYELITIS. BETTINA WARBURG, Arch. Neurol. & Psychiat. **25**:1191, 1931.

Fifteen rhesus monkeys infected with poliomyelitis were kept alive for from nineteen to thirty days after the onset of symptoms, and the spinal cord, medulla, basal ganglions, cortex and cerebellum were studied. Regardless of the mode of infection, the severest lesions were in the lumbar cord; meningitis was not a prominent feature; the blood vessels of the anterior horns were more infiltrated with lymphocytes and polymorphonuclears than elsewhere. The tissues were also infiltrated, especially in areas of severe parenchymatous destruction. Here the infiltrating cells were lymphocytes and microglia cells. The fibrous and protoplasmic astrocytes were also in evidence, but oligodendroglia cells were practically absent. Fewer anterior horn cells survived in the lumbar than in the cervical region, and in the earlier stages satellitosis was more common than neuronophagia, which was a prominent feature in the later stages.

Neuronal destruction was present mainly in the anterior horns; it was mild in Clarke's columns, in the lateral and posterior horns and in the medulla, pons, midbrain (including the substantia nigra and red nucleus) and basal ganglions. The corpus striatum was practically intact. The meninges over the cortex were more involved than over the spinal cord, while parenchymatous cortical changes were present in the anterior, frontal, precentral and posterior central areas. No relationship was noted between the extent of the perivascular infiltrations and the degenerative changes which progressively decreased cephalad. In the medulla the sensory nuclei were also involved, and in four animals that made good functional recoveries inflammatory changes persisted in the central nervous system.

GEORGE B. HASSIN.

DEATH IN ASTHMA, WITH AUTOPSY. A. M. FISHER and J. P. BECK, *J. Allergy* **2**:149, 1931.

A man, aged 32, died during an acute attack of asthma. Air had escaped into the chest cavities through small ruptures of the emphysematous lungs, which were not collapsed. The bronchioles were narrow and plugged with thick mucus containing eosinophils and Curschmann's spirals; the mucosa was thrown into folds; the basement membrane was thick and hyaline; the muscular wall was hypertrophied, and all the layers were infiltrated with plasma cells and eosinophils. The right side of the heart was hypertrophied.

ROENTGENOGRAMS IN THE STUDY OF INTRACRANIAL HEMORRHAGE IN THE NEW-BORN. F. M. B. ALLEN and H. I. McCLURE, *Arch. Dis. Childhood* **6**:97, 1931.

Intracranial hemorrhages in the new-born may be studied with advantage by stereoscopic roentgen examination after the injection of a contrast medium ("röntyum") into the carotid artery.

THE RELATION OF THE NERVOUS SYSTEM TO A PATCHY ISCHAEMIA (BIER'S SPOTS) IN ANIMALS. H. P. GILDING, *Brit. J. Exper. Path.* **12**:66, 1931.

The patchy ischemia observed after hemorrhage occurs in the absence of vasomotor control of the minute vessels concerned. The compensatory vasoconstriction that occurs after hemorrhage is a predisposing cause of the intense local ischemia observed. The production of ischemic patching in animals in denervated areas is in agreement with observations on Bier's spots in man. AUTHOR'S SUMMARY.

THE REPAIR IN VITRO OF EMBRYONIC SKELETAL RUDIMENTS AFTER EXPERIMENTAL INJURY. J. S. F. NIVEN, *J. Path. & Bact.* **34**:307, 1931.

The rudimentary bony structures of the fowl and the mouse embryo were studied on cultivation in vitro after they had been fractured or incised. The results of the reparative process, which varied according to the period of embryonic life at which the injury was made, are described.

MITOCHONDRIAL CHANGES IN OXALATE AND URANIUM NEPHRITIS. J. GOUGH, *J. Path. & Bact.* **34**:423, 1931.

In experimental oxalate and uranium nephritis in rabbits, the mitochondria are the first of the cell constituents to show alteration from the normal. Mitochondrial changes are of value in the determination of the time of onset of cellular reaction to injury. Granulation and fusion of mitochondria in the renal epithelium do not necessarily signify a permanent damage. AUTHOR'S SUMMARY.

THE MORBID ANATOMY OF MALIGNANT DISEASE OF THE MEDIASTINAL GLANDS, LUNGS AND PLEURAE. S. ROODHOUSE GLOYNE, *Tubercle* **12**:54, 1930.

Experience in the postmortem room indicates that malignant growths of the lungs, pleurae and mediastinal glands are more common now than formerly. Furthermore, there is some evidence that a change in type has occurred of late years. In postmortem examinations carried out at Victoria Park twenty years ago, the growths most commonly encountered were those situated in the mediastinal glands; now the type affecting the lungs, with secondary and often slight extension to the glands, has come much more into the picture. The histologic picture shows wide variation; growths vary from undifferentiated small round cell tumors to differentiated columnar mucous-secreting and squamous cell growths, while between the two extremes are growths of large polygonal and spheroidal cells, the so-called medullary growths. Probably nearly all are true carcinomas. The picture pro-

duced by tumors of the mediastinal glands may be summed up as one of compression, displacement, invasion and perforation of the surrounding vital structures. The malignant disease of the bronchi includes single nodular growths and diffuse bronchial carcinomatosis, while the malignant disease of the lung consists of: (1) growths of the lower lobe, generally occupying the whole lobe and producing a completely solid lobe in which the lung tissue is entirely replaced and the bronchi are occluded with growth; cavitation may follow; (2) the growths in the upper lobe resemble those in the lower lobe but are more liable to form cavities and have a predilection for infection of the tracheobronchial glands; (3) growths of the whole lung resemble those of the lower lobe, but completely obliterate interlobar fissures, fill the bronchi, are less liable to cavity formation, and exert pressure on the pulmonary vessels; (4) multiple nodules in the lung, occurring as discrete, yellowish nodules in one or more lobes of the lung; the reason for placing these in a separate class is that they suggest secondary nodules, but a primary growth is difficult to locate, and when small they are liable to be confused with actinomycosis of the lung or lymphadenomatous deposits; (5) the sclerotic type. The fifth type is described in the literature, but the author has not met a true primary, sclerotic, malignant condition of the lung. Primary malignant growths of the pleura are rare, and in all forms pleural effusion is common. The commonest concealed growths are those of diffuse bronchial carcinomatosis, the condition being obscured by bronchiectasis and general sepsis. The association of two totally different morbid conditions in one and the same lung is more common than is generally supposed. Complications of new growths of the lung are generally due to sepsis, the commonest being bronchiectasis; next in frequency is pulmonary abscess, generally in the peripheral part of the lung and usually basal. Gangrene of the lung is less common. Direct extension to the heart is an important complication of all malignant growths of the lung. No organ is immune from a secondary deposit.

H. J. CORPER.

CONGENITAL OBLITERATION OF THE LARGER BILE DUCTS. H. COBURG, Frankfurt. *Ztschr. f. Path.* **40**:281, 1930.

The case of a boy, aged 7 months, who was jaundiced since birth, is reported. At autopsy and subsequent histologic examination, it was found that the ductus choledochus was obliterated. There was no communication between the stump of the duct and the papilla of Vater, which was normal. Parts of the common hepatic and cystic ducts were obliterated. The right and the left hepatic ducts showed marked inflammatory changes. Both were filled with bile. The gallbladder was markedly diminished in size, contained no bile and revealed an inflammatory reaction throughout its wall. The liver was the seat of a typical biliary cirrhosis. The author reviews the various theories explaining such a condition; however, he arrives at no definite conclusions.

O. SAPHIR.

CALCIFICATION OF THE VASA DEFERENTIA AND THE AMPULLAE. W. SCHELLENBERG, Frankfurt. *Ztschr. f. Path.* **40**:298, 1930.

A case of calcification of the vasa deferentia and the ampullae is described in a man, aged 48. The etiology of this condition is obscure. Senile changes, tuberculosis, gonorrhea, etc., could be ruled out as etiologic factors. Repeated roentgen examinations of this region, which were done for other reasons, did not disclose the calcification. The changes were found incidentally at autopsy.

O. SAPHIR.

LIPOID DEPOSITS IN THE AORTAS OF INFANTS. N. KUBE and A. SSOLOWJEW, Frankfurt. *Ztschr. f. Path.* **40**:302, 1930.

The aortas of 114 infants were examined and stained with sudan III. None of the infants was older than 6 months. In seventy-two of the cases, lipoid deposits were found in the aorta. In one instance they were found in a stillborn infant.



They also were present in an infant 1 day old, and also in two others,  $3\frac{1}{2}$  and 9 days old, respectively. The most common locations of the lipid deposits were as follows: the upper margin of the sinus of Valsalva, the region of the commissure between the right and the posterior cusp of the aortic valve, a portion just above the left part of the sinus of Valsalva, the vicinity of the opening of the ductus arteriosus, the region where the large vessels come off from the arch of the aorta, the intima surrounding the openings of the intercostal arteries and the posterior wall of the aorta. Histologically, a varying amount of lipid droplets was seen along the elastic lamellae. The intima revealed an accumulation of macrophages, which in some instances were transformed into xanthoma cells. The belief is expressed that the lipid deposit is a purely infiltrative process, possibly the result of an imperfect lipid metabolism. There was no demonstrable relationship between acute or chronic diseases and the presence of the lipid deposits. With increasing age, these deposits not only were found more frequently, but were more severe.

O. SAPHIR.

THE FORMATION OF GIANT FOLDS IN THE MUCOSA OF THE STOMACH. H. J. SCHERER, Frankfurt. *Ztschr. f. Path.* **40**:357, 1930.

Four cases of formation of giant folds in a circumscribed area of the gastric mucosa are described. The folds were located in the posterior wall of the stomach, close to the larger curvature. There were no clinical symptoms referable to these folds. In one case, the x-ray picture led to the erroneous diagnosis of carcinoma of the stomach. Histologic examination revealed a pure hyperplasia of the mucosa. Moderate inflammatory changes, however, were encountered in two cases. The author believes that the formation of these giant folds was the result of a congenital anomaly, especially because two of the four cases also revealed other congenital anomalies. According to the author's opinion, there are no other similar cases on record. The circumscribed giant folds are thought to represent a "vitium primae formationis" in the sense of an increased "resonance" of certain cells to growth-producing irritations of various kinds, such as the influence of hormones, of nervous irritations, of inflammations and of hyperemia. It could not be determined what specific influences had caused the formation of the giant folds of the gastric mucosa in these four cases.

O. SAPHIR.

A CASE OF A RETRORECTAL LIPOMA. M. A. KOSLOW, Frankfurt. *Ztschr. f. Path.* **40**:382, 1930.

The presence of a retrorectal tumor is reported in a woman, aged 47. The tumor extended to both sides of the rectum and measured 3 by 3.5 by 12 cm. It had led to a constriction of the lumen of the rectum. Histologically, the tumor consisted of fat cells. The clinical diagnosis was rectal fissure and carcinoma of the rectum. The author suggests that in every case of stricture of the rectum one should think of a retrorectal tumor, especially lipoma.

O. SAPHIR.

DIFFUSE MYELOMA WITH AMYLOID MASSES. E. FREUND, Frankfurt. *Ztschr. f. Path.* **40**:400, 1930.

The diffuse myeloma is characterized by the absence of circumscribed nodules and by its somewhat slight osteoclastic effect. Such a tumor is described in a woman 72 years of age. The diagnosis was missed grossly, but could easily be made microscopically because of the presence of myeloma cells. Even though there was no generalized amyloidosis, amyloid masses in addition to the myeloma were found in many places, especially in the sternum and vertebrae. These masses were recognized grossly, and the impression was gained that this was a case of amyloid accumulations in healthy bones. Because of their marked calcification, the masses could be detected by the x-rays. The amyloid in many places was phago-

cytosed by giant cells. Its absence in portions gave the mass a peculiar raylike structure. Amyloid growths, contrary to diffuse myelomas, have an osteoclastic effect on the bone. The myeloma and the amyloid accumulations in this case had perforated the cortical portions of bones and the periosteum, and had invaded the adjacent cartilage and connective and fat tissue. The invasion of the periosteal region of the sixth thoracic vertebra led to a fracture of this vertebra and subsequent transverse myelitis.

O. SAPHIR.

MYXOMA OF THE HEART. A. A. WASSILIEFF, Frankfurt. *Ztschr. f. Path.* **40**:424, 1930.

Two cases of myxoma of the heart are reported. In the first case the heart showed in the region of the left auricle a gelatin-like, pinkish, papillomatous mass, filling the entire left auricle. For the most part, the mass was attached to the auricular wall. The leaflets of the mitral valve were fibrosed. Histologically, the mass showed a large amount of mucin, many small vessels and elastic and collagenous fibers. The author believes that this mass is a tumor, a myxoma, rather than a primary organized thrombus. In the second case the heart revealed a nodule somewhat smaller than a walnut, which was attached to the posterior wall of the left auricle. The surface of the nodule was smooth. It showed several yellow and red areas. The mitral valve was the seat of an acute verrucous endocarditis superimposed on a healed endocarditis. Microscopically, the nodule showed areas of hyalinization and calcification, many vascular spaces lined by endothelial cells and some iron-containing pigment. No mucin or fibrin was present. This nodule was diagnosed as an organized thrombus.

O. SAPHIR.

### Pathologic Chemistry and Physics

LACTIC ACID IN THE BLOOD IN CHILDREN. JEROME S. LEOPOLD and ADOLPH BERNHARD, *Am. J. Dis. Child.* **41**:758, 1931.

The concentration of lactic acid in the blood of twenty-three normal children varied between 9 and 18 mg. per hundred cubic centimeters of blood, the average being 14.8 mg. There was an increase of lactic acid in the blood obtained by application of a tourniquet. In forty-two children ill with various diseases (whose blood was obtained with and without tourniquets) the increase in lactic acid was from 0.2 to 6.8 mg., the average being 3.1 mg. In the blood of the normal children the average variation was 2.1 mg. A definite rise in the lactic acid content of the blood was found in cases of pneumonia with fever. In the convalescent cases without fever the values were considerably lower, but not within the normal range. In cases of rheumatic fever with cardiac involvement there was found an increase of lactic acid. Patients ill with chorea also showed an increased concentration of lactic acid in the blood. There was no relationship between the concentration of lactic acid in the blood and the blood sugar. There seems to be no quantitative relation between the concentration of lactic acid and the carbon dioxide-combining power of the blood.

AUTHORS' SUMMARY.

CHOLESTEROL AND EDEMA. JOSEPH K. CALVIN and A. H. GOLDBERG, *Am. J. Dis. Child.* **41**:1066, 1931.

The conclusions that may be drawn from a review of the literature and our experimental observations are: The blood cholesterol level in the nephrotic syndrome is practically always considerably elevated above normal, and probably does not return to normal for years, even though edema may be absent for long periods. Although the cholesterol remains above normal even during edema-free periods, it has a tendency to rise and fall with the increase and decrease of edema. Exceptions to this are not uncommon, however, and edema may appear and dis-

appear irrespective of the height of the cholesterol. The appearance or disappearance of the edema, which is usually relatively rapid when it begins, precedes the changes in the cholesterol. The cholesterol possibly may be mobilized from and at the expense of the deposits of fat in the body, for the patients are usually greatly emaciated, which becomes apparent as the edema disappears, even though the blood cholesterol remains relatively high and the intake of food is ample. The cholesterol in the nephrotic syndrome has difficulty in passing from the blood to the tissues, as the ascitic fluid has a very low cholesterol content. The blood cholesterol in the nephrotic syndrome can readily pass through the kidneys into the urine, although the hypercholesteremia antedates by a considerable period the lipiduria (Murphy). The diet has no influence on hypercholesteremia. The output of cholesterol in the bile is diminished in the nephrotic syndrome, so that apparently a real retention in the blood exists (Herrnstadt). A symptomless but definite hypoglycemia exists in nephrotic children (Knauer). It seems probable, then, that hypercholesteremia is the result of a disturbance of fat metabolism accompanying the nephrotic syndrome and not the cause or the result of edema. A further study of the relation of cholesterol to blood proteins is in progress.

AUTHORS' SUMMARY.

PLASMA PROTEIN, RED-CELL SEDIMENTATION AND SERUM LABILITY OF THE BLOOD IN TUBERCULOSIS. L. R. JONES, *Am. Rev. Tuberc.* **23**:325, 1931.

In twenty patients with pulmonary tuberculosis, as compared with twenty normal subjects, the plasma protein was found within normal limits. Fibrin was increased in nineteen, and in seven of these globulin was increased. Albumin was within normal limits in thirteen and decreased in seven. In the tuberculous group the average value of the protein quotient was 1.47, and in the normal group, 2.39. Of the tuberculous group, eleven exhibited a protein quotient within normal limits. A quantitative shift of the plasma proteins in the tuberculous patients did not bear any relationship to the type and extent of the involvement. A marked increase in sedimentation of the blood was noted in nineteen of the tuberculous patients, but the amount of the increase bore no relation to the extent or to the advancement of the disease. Increased precipitability of serum protein could not be correlated with the clinical diagnosis. Serum precipitability, though markedly increased in all of the cases of tuberculosis, was not correlated with the ratio of albumin to globulin in the plasma.

H. J. CORPER.

THE VALUE OF DETERMINATIONS OF THE IRON CONTENT OF WHOLE BLOOD. W. P. MURPHY, R. LYNCH and I. M. HOWARD, *Arch. Int. Med.* **47**:883, 1931.

Determinations of the iron content of the whole blood were made on the blood of a group of persons having essentially normal hemoglobin levels and red blood cell counts, and the figures are herein recorded. The average iron content of the blood in normal young men is 44.84 mg. per hundred cubic centimeters of blood, in normal young women, 42.48 mg., and in a group of sixty persons of both sexes and of varying age with essentially normal blood, 42.74 mg. It is suggested that a figure to be known as the "iron index" be calculated by dividing the figure for whole blood iron by the red blood cell count in millions of cells per cubic millimeter. This figure normally varies between 8 and 9, the average in this series being 8.46. In pernicious anemia, during relapse, the iron index was always found to be above 10, with a tendency to approach normal during a satisfactory remission following treatment with liver. Except in certain unusual circumstances, the iron index was found to be normal or lower than normal in chronic secondary anemia. Only in patients with anemia resulting from acute loss of blood and in certain of the patients with leukemia was the iron index above normal and in the range generally found in pernicious anemia. It is suggested from the preceding observations that the iron index is of definite value in distinguishing between per-

nicious anemia and secondary anemia in most instances, and that the constancy of the figure for whole blood iron in persons with normal blood suggests this as a satisfactory means of following the changes that may occur in the blood during treatment for anemia.

AUTHORS' SUMMARY.

THE LACTIC ACID IN THE BLOOD OF THE NEWBORN. N. J. EASTMAN and C. M. McLANE, *Bull. Johns' Hopkins Hosp.* **48**:261, 1931.

The lactic acid content of the blood of the fetus in utero is within normal limits. The lactic acid content of the blood of the infant at birth is regularly elevated, the amounts in the present series ranging from 28 to 45 mg. per hundred cubic centimeters, with an average of 35 mg. In the normal infant this increased lactic acid content of the blood at birth is due to simple diffusion from the mother, the lactic acid of whose blood at the moment of delivery is increased as the result of the muscular activity incident to labor. In the asphyxiated infant a very definite endogenous production of lactic acid occurs, the lactic acid of the blood rising above that in the maternal blood to reach a concentration as high as 90 mg. per hundred cubic centimeters. The high concentration of lactic acid of the blood exhibited by asphyxiated infants makes it seem probable that asphyxia neonatorum is associated with considerable acidosis; but in the present state of knowledge one hesitates to draw conclusions as to how important a factor it may be.

AUTHORS' SUMMARY.

LEAD IN FECES AND URINE. F. FRETWURST and A. HERTZ, *Arch. f. Hyg.* **104**:315, 1930.

The average normal excretion of lead is 0.5 mg. per kilogram of feces and 0.03 mg. per liter of urine. The amounts excreted by lead workers with no symptoms of poisoning may be only slightly higher. Persons with definite symptoms of lead poisoning may excrete as much as 2 mg. per kilogram of feces and 0.07 mg. per liter of urine.

ARTHUR LOCKE.

THE CATALASE ACTION AND GLUTATHIONE CONTENT OF ERYTHROCYTES IN ANEMIAS. EMERICH BACH and ERNST BACH, *Biochem. Ztschr.* **236**:174, 1931.

The cold reduction of blood is mainly due to glutathione. In true pernicious anemia, the relative catalase and glutathione contents of the erythrocyte are increased. In secondary anemias with preceding cachexia, the catalase and glutathione contents of the erythrocyte are not increased. In acute losses of blood, the relative glutathione content is increased, while the relative catalase content does not change. The value catalase-erythrocytes is independent of macrocytosis and hyperchromatism. The same conditions that cause an increased respiration of the erythrocyte produce also an increase in its catalase and glutathione content. This is interpreted as evidence that catalase is involved in cellular respiration.

WILHELM C. HUEPER.

PHOSPHATIDES AND AMINES OF THE BLOOD IN DISEASES OF THE KIDNEY AND THEIR RELATIONS TO HYPERTENSION. K. HOESCH, *Klin. Wchnschr.* **10**:881, 1931.

With the marked hypertension in malignant sclerosis, fixed hypertonicity and chronic nephritis, the amino-nitrogen is increased. The amino-nitrogen is contained to some extent in an ether-soluble uramino acid and base, which are not related to the phosphatides, and which occur in a free state. The increase in cephalin in malignant sclerosis may be due to decomposition processes (lecithin  $\rightarrow$  cephalin). There is no disturbance in the phosphatide content.

AUTHOR'S SUMMARY.

A METHOD FOR THE DETERMINATION OF FIBRIN, GLOBULIN AND ALBUMIN IN BLOOD PLASMA. HUGO THEORELL and GÖSTA WIDSTRÖM, *Ztschr. f. d. ges. exper. Med.* **75**:692, 1931.

The method described permits reliable determinations to be made on as little as 1 cc. of plasma. The method employs nitrogen determinations with hypobromite, after recalcification from citrated plasma in the case of fibrin, and after salting out with magnesium sulphate in the case of globulin and albumin.

PEARL ZEEK.

### Microbiology and Parasitology

PERNICIOUS MALARIA. ETTORE MARCHIAFAVA, *Am. J. Hyg.* **13**:1, 1931.

Evidence is presented for the existence of three different types of malaria and for their endoglobular position in the red blood corpuscle. Pernicious malaria occurs solely as a result of infection with *Plasmodium falciparum*, never in winter or early spring and only as recent infections or early relapses. The proliferation of endothelial cells in the blood stream has its diagnostic importance, as well as being a measure of bodily defense. True tertian fever occurs in these cases, unless the picture is altered by a mixed or multiple infection or by atypicality in the time of the occurrence of sporulation. The sporulation in this type of malaria occurs in the visceral circulation, and the gametocytes appear in the peripheral circulation usually several days after the onset of the fever. The greater pathogenicity of *P. falciparum* is explained by its shorter period of incubation, more frequent multiplication, greater number of spores, greater toxicity and its resistance to quinine. The clinical and pathologic features of pernicious malaria are presented, as well as the rational therapeutic measures.

P. H. GUINAND.

BACILLI OF THE GENUS *HEMOPHILUS* WITH REGARD TO THE X AND V GROWTH FACTORS. LUCILE R. ANDERSON, *Am. J. Hyg.* **13**:164, 1931.

*Bacillus influenzae*, hemolytic and nonhemolytic, requires both the X factor and the V factor under aerobic conditions. The four types of hemophilic bacilli studied do not require the X factor for growth under anaerobic conditions, but require the V factor. From their cultural reactions it would appear that the bacilli of influenza carry on a normal existence in mediums containing only the V factor under anaerobic conditions. Not one of thirteen anaerobes tested reacted positively to the benzidine test for peroxidase; of forty-four aerobes tested, all but four, two of which were streptococci, gave a positive reaction. *Bacillus influenzae*, hemolytic and nonhemolytic, and *Hemophilus canis* do not produce peroxidase. *Bacillus para-influenzae* produces peroxidase under both aerobic and anaerobic conditions. Banana reacts positively to the benzidine test for peroxidase. All evidence appears to substantiate Fildes' view that the X factor is associated and identical with peroxidase. Evidence is submitted supporting the idea of Davis that there is interaction of the X and V factors.

Note: The X and V factors are both present in hemoglobin. The X factor is heat stable and is closely associated with the iron-containing pigment. The V factor is heat labile, and its nature is not well understood.

AUTHOR'S SUMMARY.

THE CULTIVATION OF *BALANTIDIUM COLI*. EUGENE SCHUMAKER, *Am. J. Hyg.* **13**:281, 1931.

*Balantidium coli* is facultatively anaerobic. It multiplied as rapidly under strictly anaerobic conditions as under aerobic conditions, but a condition of strict anaerobiasis was not more favorable to growth than an aerobic condition. The growth of *Balantidium* is not inhibited by an oxygen pressure of 18 pounds (8.2 Kg.) per square inch when this pressure is maintained for thirty-two hours. Tropho-

zoites of *Balantidium* from an experimentally infected rat were not killed by an oxygen pressure of at least 30 pounds (13.6 Kg.) per square inch maintained over a period of seventy-two hours. Inulin may serve, to a slight extent, as a nutrient carbohydrate for *Balantidium* in cultures. Potato, wheat, corn, buckwheat and rice starches served equally well for the cultivation of *Balantidium*. Arrow-root starch was also used successfully in cultures. The parasite multiplied at temperatures from 23 to 41 C.; its optimum seemed to be from 37 to 39 C. Multiplication of the organisms was not markedly inhibited at 41 C., but was greatly reduced at and below 34 C. *Balantidium* was cultivated successfully for thirty-eight days on a modified medium in which only 3 per cent of horse serum was used.

## AUTHOR'S SUMMARY.

THE RELATION OF TEMPERATURE AND HUMIDITY TO THE COURSE OF A B. ENTERITIDIS INFECTION IN WHITE MICE. I. J. KLIGLER and L. OLITZKI, *Am. J. Hyg.* **13**:349, 1931.

White mice from the same stock and of the same age infected with the same number of organisms of the species *Bacterium enteritidis* and kept at different temperatures and humidities react differently to the infecting microbes: At a low temperature (from 10 to 12 C.) and a high relative humidity (90) the infection is more severe than at higher temperatures (20 and 30 C.) and the same humidity. The mortality is the same, but the incidence of abscesses of the liver is higher. At 30 C., the mice kept at a relative humidity of 90 are more severely affected than those kept at a relative humidity of 35. At a humidity of 35 and a temperature of 30 C., the development of the infection is more rapid, but it is mild or unapparent, clears up rapidly and leads to relatively few deaths in comparison with infection at a relatively high humidity. At 20 C., a higher humidity seems more favorable than a lower one, but the differences are not marked. It appears that the critical factor is not temperature alone nor humidity alone, but the combination of the two—probably constituting the so-called "effective temperature."

## AUTHORS' SUMMARY.

LOCATION OF DENGUE VIRUS IN THE BODY OF MOSQUITOES. R. L. HOLT and J. H. KINTNER, *Am. J. Trop. Med.* **11**:103, 1931.

Dengue in mosquitoes of the genus *Aedes* does not involve any particular part of the insect, but is septicemic. It is believed that the so-called period of "maturation of the virus" of from eleven to fourteen days before the mosquito becomes infective to man is merely the time required for the virus to become of such concentration in the insect as to allow the injection of an infective dose into man. It is believed that this, with the fact that the virus may be transferred from infected to normal mosquitoes for at least three serial transfers, in a form undiminished in its ability to infect man, by feeding normal mosquitoes on a suspension of infected mosquitoes ground up in normal blood, mitigates strongly against the theory held by certain investigators that a cyclic phase in the life of the virus is accomplished in *Aedes*. It seems that this mosquito is merely a means of transmission from man to man and is not necessary to the continued existence of the virus.

## AUTHORS' SUMMARY.

ATTEMPTS TO PRODUCE BRONCHOMONILIASIS IN MONKEYS. HOBART A. REIMANN and TIMOTHY J. KUROTCHKIN, *Am. J. Trop. Med.* **11**:151, 1931.

Intratracheal, intravenous and direct inoculation of *Monilia tropicalis*, isolated from a fatal case of bronchomoniliasis, into the lungs of monkeys failed to induce pulmonary infection. Two normal animals were inoculated intratracheally in attempts to induce a primary infection, without success. In the other monkeys efforts were made to reduce the resistance of the lungs by injection of inert foreign bodies, by actual laceration of the pulmonary tissues and by intravenous

injection of chaulmoogra oil, in the hope that *Monilia* would grow as a secondary invader in the damaged tissues. Several suggestions to explain the failure to produce lesions may be mentioned: 1. Monkeys may be resistant to infection with *Monilia*, or the strain used may have been avirulent for these animals. 2. The lung may not have been damaged severely enough to permit the organisms to gain a foothold. 3. The monilias may not have reached the damaged tissue. 4. There was no obstruction in the bronchi to prevent the evacuation of the inoculum. 5. The monilias inoculated were from young cultures, i. e., they were in the yeast-like stage of their life cycle. It has been suggested that in the mycelial, or older, stage the organisms are more resistant and invasive. In another communication, the formation of tubercle-like nodules in the traumatized lungs of rabbits following the inoculation of old cultures of *Monilia tropicalis* is reported.

AUTHORS' SUMMARY.

THE INTRATRACHEAL INOCULATION OF MONKEYS WITH PNEUMOCOCCI. G. W. STUPPY, I. S. FALK and M. A. JACOBSON, *J. Prev. Med.* 5:81, 1931.

Intratracheal inoculation of monkeys of the species *Macacus rhesus* and *Cebus capucinus* with varying amounts of virulent pneumococci of type I did not result in lobar pneumonia, although in most cases it brought about an increase in temperature and leukocytic reactions, with pneumococcal septicemia. Recovery usually occurred within a week after inoculation. Only two of thirteen monkeys died of the pneumococcal infection. The lungs were found to be normal, except for an increase in the number of polymorphonuclear leukocytes in the interstitial tissue, blood vessels and bronchi. Intratracheal inoculation of the monkeys with cultures of low virulence, followed by cultures of progressively increasing virulence, gave rise to a slight increase in temperature and in the number of leukocytes, but no septicemia. The absence of septicemia indicated that some immunity had been produced by the previous inoculations of pneumococci of lower virulence. On the whole, *Macacus rhesus* and *Cebus capucinus* appeared to be highly resistant to pneumococcal infection.

AUTHORS' SUMMARY.

INTRABRONCHIAL INSUFFLATION OF PNEUMOCOCCI IN RABBITS. G. W. STUPPY and I. S. FALK, *J. Prev. Med.* 5:89, 1931.

In rabbits intrabronchial insufflation of pneumococci of uniformly high virulence gave rise to a bronchopneumonia that usually caused death in from two to five days, with septicemia and a generalized distribution of pneumococci in the lungs. In some animals there was acute inflammation of the interstitial tissue of the lung and perivascular and peribronchial lymphangitis. Suppurative bronchitis and pleuritis were only occasionally seen. The factor of resistance to pneumococcal infection among individual rabbits is of importance in determining the type of lesions produced by the introduction of pneumococci into the lung. Attempts to lower resistance by chilling were not successful, as the chilling did not appreciably increase the rabbits' susceptibility to pneumococci, but did apparently predispose them to spontaneous infections, which in the control rabbits (chilled, but not inoculated) were frequently fatal. The lesions induced in the lungs of the rabbits by insufflation of pneumococci of types I, II and III, and of the same degree of virulence (as measured in white mice), were on the whole similar. The virulence of the pneumococcus employed, rather than its serologic type, would appear to be the important factor in the production of infection.

AUTHORS' SUMMARY.

EXPERIMENTAL POLIOMYELITIS. P. H. HARMON, H. J. SHAUGHNESSY and F. B. GORDON, *J. Prev. Med.* 5:115 and 139, 1931.

Prior to the onset of experimental poliomyelitis in rhesus monkeys following intracerebral inoculation of virus, changes occur in the body temperature, in the numbers of polymorphonuclear neutrophilic leukocytes in the blood and in the

cell and globulin contents of the cerebrospinal fluid. These changes are analogous to those that occur in poliomyelitis in man. The first constant alteration detectable in the preparalytic stage of the experimental disease is the change in the body temperature. In many, but not all, monkeys the preparalytic increase in the numbers of neutrophilic leukocytes is coincident with the rise in the body temperature; in a few cases the change in the leukocyte count occurs a day or two after the rise in the temperature. Changes in the spinal fluid occur definitely one or two days after the changes in the temperature, except in a few animals in which the two changes are observed simultaneously. Alterations in the velocity of the sedimentation of the erythrocytes occurred only after the onset of paralysis, usually after the monkey was prostrate from an extension of the disease.

Our criterion for abortive poliomyelitis in monkeys is absence of paralysis, but presence of symptoms and of changes in the spinal fluid or of symptoms and of an increase in the body temperature and in the numbers of neutrophilic leukocytes, the symptoms, the changes in the spinal fluid and the increase in the temperature and in the numbers of leukocytes occurring after a period of incubation and resembling those observed in the preparalytic stage of the ordinary form of the disease. Following 555 intracerebral inoculations in 350 monkeys, only 10 cases of abortive poliomyelitis occurred. We found 7 instances of "missed infection," in which a certain dilution of a virus caused no paralysis in a monkey, although a monkey receiving a higher dilution became paralyzed. Some of these may have been cases of abortive poliomyelitis, although they did not conform to our criterion. Two cases in which paralysis occurred thirty-three and fifty-one days, respectively, after inoculation were probably cases of relapsing poliomyelitis. Abortive and relapsing poliomyelitis and "missed infections" seem to be the result of differences in susceptibility in individual monkeys, rather than of mild infection with an attenuated virus.

AUTHORS' SUMMARIES.

EFFECTS ON GUINEA-PIGS OF FILTRATES OF TUBERCULOUS SPUTUM. A. LARSON, *J. Prev. Med.* 5:161, 1931.

Filtrates of sputum from tuberculous patients, containing numerous tubercle bacilli, were inoculated subcutaneously into forty-three guinea-pigs, ten receiving a Seitz filtrate, twenty-two a Berkefeld filtrate and eleven a filtrate passed through both Seitz and Berkefeld filters. None of these animals showed the cachexia which is said to be characteristic of the more usual form of infection from filtrates. Neither was there any enlargement of the inguinal lymph glands at the point of inoculation. At autopsy no lesions suggestive of tuberculosis were found. Smears made from the tracheobronchial and retroperitoneal lumbar lymph glands showed no acidfast rods or granules. Control guinea-pigs inoculated with small doses of unfiltered sputum from each patient died with generalized tuberculosis. In these results there is no evidence that tuberculous infection is caused by Seitz or Berkefeld filtrates of sputum. When considered in connection with the numerous negative results of others, these results make it probable that the positive signs of infection that have been reported were due to some other factor than a filtrable virus. The silver plating of the cup of the Seitz filter exerts no appreciable oligodynamic action on suspensions of tubercle bacilli allowed to remain within it for eighteen hours.

AUTHOR'S SUMMARY.

LETHARGIC ENCEPHALITIS: THE GLASGOW EPIDEMIC OF 1923. ASHIE MAIN, *J. Hyg.* 31:162, 1931.

The main features of the Glasgow epidemic of encephalitis of 1923 are portrayed in this review. The review is in no sense an intimate neurologic study. The author was sought, rather, to furnish a general impression of the clinical phenomena as they first presented themselves, and to follow the picture as it unfolded itself in the succeeding phases one year and five years later. In this way there have been thrown into relief the characteristics that distinguish epidemic encephalitis



from poliomyelitis and the encephalitis of influenza. A critical summary of the sequelae reveals and illustrates the hypothesis that they are to be explained, not on the assumption of a progressive lesion of the nervous system, such as dementia paralytica, but on the assumption of an initial and permanent damage to the complicated neural mechanism of the midbrain, hypothalamus and basal nuclei. The consequent instability of the mechanism renders it prone to give expression to various forms of disability, depending on ill-defined proclivities, one of which is associated with the age of the patient at the time of the initial attack. However inscrutable its origin and however incalculable its course or obscure the conglomeration of its individual manifestations, there is no difficulty in recognizing the scar it leaves on the health of the community.

FROM THE AUTHOR'S SUMMARY.

### Immunology

THE NEUTRALIZATION OR DESTRUCTION OF DIPHTHERIA TOXIN BY TISSUE.  
A. WADSWORTH and E. N. HOPPE, *J. Exper. Med.* **53**:821, 1931.

As determined by the intracutaneous test in guinea-pigs, diphtheria toxin is not altered in the presence of cardiac tissue obtained from the fetal or from the adult heart of the guinea-pig. Tissue cultures were apparently uninjured by the presence of the toxin in the dilutions used in these experiments, and, when washed with embryo extract after removal of the diluted toxin, continued to grow. Embryonic guinea-pig cardiac muscle tissue growing in cultures in vitro possesses the power of neutralizing, binding or destroying diphtheria toxin so that it is no longer toxic for normal guinea-pigs. Such neutralization takes place through the intervention of growing tissue and is a property that is lacking in similar surviving tissue not in a state of cultivation. Thus, it appears that the living, growing cells of the tissues neutralize or destroy limited quantities of toxins; only when the quantity of toxin exceeds a certain limit is its action injurious.

AUTHORS' SUMMARY.

ACTIVE IMMUNIZATION AGAINST POLIOMYELITIS IN MONKEYS. M. BRODIE and A. GOLDBLOOM, *J. Exper. Med.* **53**:885, 1931.

A combination of poliomyelitis virus and specific human serum is effective for the production of active immunity. For each gram of active virus given intradermally as an emulsion, 6 cc. of human immune serum, injected subcutaneously, was required in our experiments to protect a monkey from paralysis. Some degree of active immunity was induced. Immunity, without symptoms of the disease, was secured when the serum was given at the time of inoculation, or within three days preceding or following inoculation of the virus. For the production of immunity, virus, preceded by serum administration, is probably less effective than when it is given simultaneously with, or before, the injection of serum. The virus neutralization test is more sensitive than the direct intracerebral test for determining the production of immunity.

AUTHORS' SUMMARY.

LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES: PASSIVE IMMUNITY.  
G. SHWARTZMAN, *J. Exper. Med.* **54**:1, 1931.

It has proved possible to elicit passive immunity to *B. typhosus* reacting factors by means of normal and immune homologous neutralizing antibodies. The in vivo serum protection against these factors followed the law of multiple proportions. There was observed a considerable loss of antibodies from the blood stream. Passive immunity was best obtained when the immune serum was injected intravenously half an hour before the intravenous injection of the reacting factors. It was possible to prevent the occurrence of the local skin reaction by an intravenous injection of serum after the intravenous injection of the reacting factors, provided

that the serum dose was very large and that the serum injection was made immediately after the filtrate injection. A number of experiments clearly demonstrated the interesting fact that the greater the amount of antiserum injected intravenously, the more efficient was the *in vivo* neutralization, in a ratio distinctly greater than the quantitative increase of serum. It is suggested that there may be a practical value of the observation in relation to serum therapy.

AUTHOR'S SUMMARY.

INTRACUTANEOUS PNEUMOCOCCUS INJECTION. J. FREUND, *J. Exper. Med.* **54**:171, 1931.

Young and adult rabbits react differently to intracutaneous injection of virulent pneumococci. In adult rabbits a very extensive inflammation develops at the site of infection, and bacteremia and death occur only in a relatively few rabbits. Young rabbits fail to develop extensive inflammation and die with bacteremia. It is probable that the fate of the animals is influenced by the capacity to develop inflammation at the site of injection of pneumococci.

AUTHOR'S SUMMARY.

LOCAL ORGAN HYPERSENSITIVENESS (RABBIT'S EYE). D. and B. C. SEEGAL, *J. Exper. Med.* **54**:249 and 265, 1931.

Rabbit eyes sensitized with guinea-pig red blood cells or fresh egg white respond with an inflammatory reaction following the intravenous injection of the homologous, but not the heterologous, antigen. A multiple antigen containing minute amounts of separate antigens is sufficient to cause this sensitiveness. The reaction has been obtained as long as eight months after sensitization. Repeated daily injections of a single antigen produces no reaction after the first few days, while the injection of various antigens on succeeding days produces inflammation. Permanent desensitization does not occur after eight months unless large doses of antigen are used. The reaction is not due to an initial tissue injury. In the actively sensitized rabbit eye a sterile inflammation may develop after the introduction of the homologous antigen into the gastro-intestinal tract.

EDNA DELVES.

COPPER AND IRON IN IMMUNITY. A. LOCKE and E. R. MAIN, *J. Infect. Dis.* **48**:419, 1931.

Neurotoxins appear to be dispersions of bacterial protoplasm containing fragments of a positively charged respiratory substance having copper as the predominant catalyst. They resemble respiratory enzymes of the oxydase type and the proteases of the  $p_H$  8 crepsin type in being inactivated by sodium cyanide and cysteine. The hemotoxins appear to contain fragments of negatively charged respiratory substance having the ferrous iron as the predominant catalyst. They resemble the respiratory enzymes of the dehydrogenase type and the proteases of the  $p_H$  4 papain type, which are not inactivated by sodium cyanide or cysteine, but are inactivated by the cupric ion.

AUTHORS' SUMMARY.

LOCAL SKIN REACTIVITY (SHWARTZMAN'S REACTION) TO PNEUMOCOCCUS "FILTRATES." E. J. COPE and K. M. HOWELL, *J. Infect. Dis.* **48**:570, 1931.

Filtrates of pneumococci which were not disintegrated gave no Shwartzman skin reactions, while solutions of pneumococci dissolved in bile produced such reactions. No skin reactions were obtained with egg white, dilute bile, ascites broth, spinal fluid and horse serum. Human serum and a mixture of bile, ascites broth and salt rarely produced a reaction. Group specificity of pneumococci was demonstrated in all but one case. The phenomenon of local skin reactivity appears more valuable than agglutination in determining pneumococcal types. Whether each type and strain of pneumococcus has a specific toxic substance, has to be determined.

EDNA DELVES.

SIMULTANEOUS MULTIPLE IMMUNIZATION. L. HEKTOEN and A. K. BOOR, J. Infect. Dis. **48**:588, 1931.

The rabbit is capable of producing many specific precipitins in response to the simultaneous introduction of many single antigens.

AUTHORS' SUMMARY.

SPECIFICNESS OF HEMOGLOBIN PRECIPITINS. L. HEKTOEN and A. K. BOOR, J. Infect. Dis. **49**:29, 1931.

At times antihemoglobin precipitin serum, obtained in response to a pure, single antigen, may react with other hemoglobins than the one that served as the antigen. Such extraspecific action may be overcome by diluting the serum. It appears that hemoglobin may cause responses of wider relationship than those of species.

AUTHORS' SUMMARY.

EXPERIMENTS BEARING ON ACUTE RHEUMATIC FEVER. B. J. CLAWSON, J. Infect. Dis. **49**:90, 1931.

Rabbits made hypersensitive to streptococci can be desensitized by intravenous administration of a streptococcic vaccine. Injections with doses of streptococci that produce marked lesions in hypersensitive animals have little or no effect on vaccinated hypersensitive animals. This protective phenomenon is not strictly type specific, but seems probably species specific. It does not seem to be in the category of a reaction to nonspecific protein. Protection is uniformly associated with a high titer of agglutination, while hypersensitiveness is not. The use of intravenous vaccine treatment in acute rheumatic fever is suggested.

AUTHOR'S SUMMARY.

QUANTITATIVE ASPECTS OF IMMUNITY REACTIONS. J. MARRACK and F. C. SMITH, Brit. J. Exper. Path. **12**:30 and 182, 1931.

The compound of horse-serum pseudoglobulin and its antibody is slightly soluble in 0.9 per cent NaCl solution. The amount of precipitate obtained from mixtures of antigen and antibody in optimum proportions is not affected by moderate variations in the concentration of electrolytes. When antigen is in excess and the volume large, the amount is greatly increased by the addition of 0.1 N BaCl<sub>2</sub>. This we consider to be due to the neutralization of the negative charge of an insoluble suspension. The amount of precipitate is unaffected by the addition of nonspecific proteins. The precipitate is composed of protein; no substances soluble in fat solvents were detected.

The ratio of antigen to total protein in the precipitate formed in a precipitin reaction increases when increasing amounts of antigen are added to a given amount of antibody. The union of the antigen-antibody compound with more antigen or antibody, when either is added in excess, is not due to nonspecific adsorption. The total amount of precipitate from a given amount of serum and the ratio of antigen to total protein are higher with strong serums than with weak serums. The amount of precipitate and rate of flocculation may be increased by the addition of nonspecific proteins, but the ratio of antigen to total protein in the precipitate is little altered. It is inferred that nonspecific proteins may increase the rapidity and completeness of flocculation of the antigen-antibody compound, but are very little adsorbed.

AUTHORS' SUMMARIES.

IMMUNOLOGICAL DIFFERENCES BETWEEN STRAINS OF POLIOMYELITIC VIRUS. F. M. BURNET and JEAN MACNAMARA, Brit. J. Exper. Path. **12**:57, 1931.

A poliomyelitic virus derived from a child dying in Melbourne has shown distinct immunologic differences from the Rockefeller Institute "mixed virus" strain both in cross-immunity experiments and by neutralization tests *in vitro*. Three instances are described of monkeys that contracted a typical fatal infection after

injection of the heterologous virus, despite the fact that some weeks previously they had suffered a typical attack of experimental poliomyelitis.

## AUTHORS' SUMMARY.

A SPECIFIC PRECIPITATING POLYSACCHARIDE FROM *B. DYSENTERIAE* (SHIGA).  
W. T. J. MORGAN, Brit. J. Exper. Path. **12**:62, 1931.

A method is described for the isolation of a polysaccharide from the smooth variant of *B. dysenteriae* (Shiga). The substance obtained by this method gives specific precipitation with the homologous immune serum up to a dilution of 1 in 6,000,000. The intravenous inoculation of this polysaccharide into a rabbit did not give rise to the production of demonstrable antibodies.

## AUTHOR'S SUMMARY.

AGGLUTININS OF TICK-BITE FEVER AND SPORADIC TYPHUS IN SOUTHERN AFRICA. ADRIANUS PIJPER and HELEN DAU, Brit. J. Exper. Path. **12**:123, 1931.

The typhus-like disease in Southern Africa, which we propose to call tick-bite fever, produces agglutinins for *B. proteus*, OX 19, OX 2 and X Kingsbury. Typhus in South Africa produces agglutinins for OX 19 and OX 2, but not for X Kingsbury. Guinea-pigs infected with either tick-bite fever or typhus develop agglutinins for X Kingsbury, but not for OX 19 nor for OX 2. All these agglutinins appear to become more active if one lets the serums stand for some days. This phenomenon of "temporary inhibition" may explain certain discrepancies observed in agglutination reactions with X-strains. In tick-bite fever agglutinins appear late in the disease.

## AUTHORS' SUMMARY.

OBSERVATIONS ON *SALMONELLA* AGGLUTINATION AND RELATED PHENOMENA.  
P. B. WHITE, J. Path. & Bact. **34**:325, 1931.

Saline extracts of heated *Salmonella* bacilli and the carbohydrate haptenes set free from these organisms by antiformin have the same powers of fixing somatic agglutinins as have the bacillary bodies from which they are derived. On the other hand, carbohydrate haptenes prepared by the author's acetic acid method lack appreciable ability to bind the agglutinins of much diluted antiserum.

## AUTHOR'S SUMMARY.

AUTOHAEMAGGLUTINATION. W. BOXWELL and J. W. BIGGER, J. Path. & Bact. **34**:407, 1931.

A case of autohemagglutination which occurred in a woman, aged 65, who suffered from anemia and an atypical leukemia, is described. Her serum agglutinated her own cells and the cells of the four blood groups rapidly and markedly at air temperature but not at body temperature. The literature is reviewed, and it is concluded that up to the present only 22 authentic cases of the condition have been described. A new definition of autohemagglutination is suggested as follows: Autohemagglutination is a clumping of erythrocytes into irregular masses, visible to the naked eye, occurring in the presence of the individual's own serum, without bacterial action, at air temperature and reversible at body temperature.

## AUTHORS' SUMMARY.

SEROLOGICAL CLASSIFICATION OF MONILIAS. K. STONE and L. P. GARROD, J. Path. & Bact. **34**:429, 1931.

The complement-fixation and precipitin reactions have been applied to the study of monilias. All monilias cultivated from cases of thrush were found identical

when examined by these methods. Of 14 strains of *Monilia* found in various types of pathologic material and examined by both these methods, 12 were found to be identical with the *Monilia* of thrush. The application of the same tests to 10 named types of *Monilia* (Castellani) indicates that 6 of these are identical with the *Monilia* of thrush.

AUTHORS' SUMMARY.

ACTION OF CONGO RED ON STREPTOCOCCAL AND *B. WELCHII* HEMOLYSIN. J. GORDON, *J. Path. & Bact.* **34**:439, 1931.

Certain concentrations of solutions of congo red neutralize the hemolysins of *Streptococcus hemolyticus* and *B. welchii*. These neutralizations are reversible; in the case of the streptococcus the addition of cuprammonium artificial silk adsorbs the congo red and liberates the hemolysin. This method cannot be used with *B. welchii*, as the hemolysin is destroyed by artificial silk. Reversibility with both the hemolysins of *S. hemolyticus* and *B. welchii* can be shown by the adsorption of congo red on ox serum. Reversibility is best effected where the concentration of congo red is just sufficient to neutralize the hemolysin. Congo red does not neutralize the hemolytic activities of solutions of saponin, sodium taurocholate or sodium ricinoleate.

AUTHOR'S SUMMARY.

STAPHYLOCOCCUS TOXIN ANATOXIN AND ANTITOXIN. F. M. BURNET, *J. Path. & Bact.* **34**:471, 1931.

*Staphylococcus* toxin is rapidly detoxicated by formaldehyde at 37 C. The atoxic preparation (anatoxin) is an effective immunizing agent and is capable of binding antitoxin in vitro. The binding power of a freshly detoxicated preparation is, within the limits of experimental error, accurately half that of the toxin of origin. By the use of anatoxin it can be shown that the toxin-antitoxin and anatoxin-antitoxin reactions are almost completely irreversible within a minute or two of mixing. From the partition of antitoxin between toxin and anatoxin it is deduced that both reactions are of the same molecular order. The toxin-antitoxin neutralization curve for staphylo toxin is described. A discussion of the toxin-antitoxin reaction in general leads to the conclusion that the reaction is primarily a true stoichiometric union modified by adsorption of the constituent on to aggregates of toxin-antitoxin molecules.

AUTHOR'S SUMMARY.

NEW SEROLOGICAL TYPE OF SALMONELLA. P. H. LESLIE and A. G. SHERA, *J. Path. & Bact.* **34**:533, 1931.

A new type of *Salmonella* organism has been isolated by blood culture from a human case of clinical paratyphoid fever. Its antigenic structure, so far as the analysis has been carried, may be expressed in symbols (Bruce White 1926 and 1929) as follows: Specific phase =  $D_1, D_2 +$  a minor factor; group phase = G,  $E_1$  and  $E_2 +$  minor factors; "O" antigen = III. It is proposed to call this type *Salmonella eastbourne*.

AUTHORS' SUMMARY.

FRACTIONS OF DIFFERENT ANTITOXIC QUALITY FROM THE SAME SERUM. M. BARR and A. T. GLENNY, *J. Path. & Bact.* **34**:539, 1931.

A diphtheria antitoxic plasma giving an in vivo value of 950 units and in vitro value of 625 units (ratio, 1:52) has yielded, by successive precipitations with ammonium sulphate, a series of globulin fractions of different antitoxic quality. The ratio of the fractions precipitated by the least amounts of ammonium sulphate is greater than that of the original plasma. The ratio decreases progressively throughout the series from 2.69 in the first fraction to 0.93 in the last.

AUTHORS' SUMMARY.

STUDIES OF PROTECTION AGAINST TUBERCULOSIS. A. STANLEY GRIFFITH, Medical Research Council, Special Report Series no. 152, 1931.

The strain of B C G used in these experiments can produce local lesions in the rhesus monkey, but these are always benign and do not lead to generalization. B C G given by the mouth can pass through the mucous membrane of the alimentary canal into the adjacent glands and also gain access to the blood-stream. Vaccination with B C G, whether by feeding or by injection, has failed to give to monkeys the complete protection against tuberculosis reported by Wilbert, but in some instances may have produced a low grade of relative immunity.

AUTHOR'S CONCLUSIONS.

THE VARIOLA-VACCINIA FLOCCULATION. J. CRAIGIE and W. J. TULLOCH, Medical Research Council Special Report Series, no. 156, 1931.

The variola-vaccinia flocculation reaction is specific and has diagnostic value. The test is made best with antiserum of constant concentration and extract of varied concentration. Treatment of the crusts with ether before extraction with salt solution is of advantage. The reaction is not due to agglutination of secondarily infecting bacteria.

FROM AUTHORS' CONCLUSIONS.

EXPERIMENTAL STUDIES ON FORMATION OF AGGLUTININS AND PRECIPITINS. T. H. AMAKO, Ztschr. f. Immunitätsforsch. u. exper. Therap. 70:400, 1931.

Rabbits having a high normal antibody titer against certain bacteria responded with a stronger antibody production when immunized with those bacteria than with others. In rabbits revaccinated after an interval, isovaccines had a better immunizing effect than heterovaccines; the antibody response was proportionate to the genetic relationship of the organisms of the revaccination to the bacteria of the original immunization. The withdrawal of blood had a stimulating effect on the titers of agglutinins and precipitins. The development of both antibodies was parallel, but agglutinins appeared somewhat earlier and were more heat-resistant. The absorption of one of the antibodies did not affect the titer of the other.

I. DAVIDSOHN.

VARIABILITY, NOT TYPE SPECIFICITY, IN THE GROUP OF THE PARATYPHOID-FOOD POISONING BACTERIA. HANS MEYER and H. OTSU, Ztschr. f. Immunitätsforsch. u. exper. Therap. 70:413, 1931.

Following the procedure of Andrewes, it was possible to isolate strains of *Bacillus paratyphosus* Breslau, which were agglutinated only by a Breslau antiserum and not by other serums, and which were unable to absorb Schottmueller agglutinins. However, immune serum produced with such a specific culture agglutinated also nonspecific Breslau strains and even a few Schottmueller strains. The agglutinins for the different strains could be absorbed separately. The specific strain or "phase" is not an unchangeable characteristic, but a reversible variant of a dominant character with a recessive nonspecific phase, which can reappear any time in the culture or in the antibodies of the immune serum. Therefore, the use of serums produced according to Andrewes' procedure is not reliable for diagnostic purposes.

I. DAVIDSOHN.

A MODIFICATION OF SERODIAGNOSIS OF SYPHILIS WITH INACTIVATED SERUM. TSIEN-YUNG TSÜ, Ztschr. f. Immunitätsforsch. u. exper. Therap. 70:445, 1931.

Normal rabbit serum is used as source of hemolytic antishoop amboceptor and of complement. Rabbits of equal weight have, in the author's experience, almost equal complement and amboceptor titers, which precludes the necessity of preliminary titration. The patient's serum is inactivated. Comparative tests with other methods showed very good agreement.

I. DAVIDSOHN.

THE SPECIFICITY OF THE SO-CALLED DIAGNOSTIC BACTERIOPHAGES. MARIO E. MASSA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:525, 1931.

A check of the work of Sonnenschein who claimed to have produced specific diagnostic bacteriophages for the Bacterium of Schottmueller, Bacterium dysenteriae and Bacterium typhosum. While some of these bacteriophages preferred certain of the aforementioned bacteria, they invariably acted also on the variants of the other bacteria. The nonspecific lysosensitive variants make the use of bacteriophages for differential diagnosis unreliable.

I. DAVIDSOHN.

INTENSIFYING THE PROPHYLAXIS OF RABIES. OTTO HERRMANN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:536, 1931.

The improvement of mortality records following vaccinations against rabies is due mainly to the broadening of indications for the treatment. The intensifying of the treatment by increasing the dosage and shortening the period to from three to six days is not advisable. Stretching of the same dose over a longer period is preferable.

I. DAVIDSOHN.

IMMUNITY IN TUBERCULOSIS. E. HEDVALI, *Ztschr. f. Tuberk.* **60**:97, 1931.

Bactericidal antibodies were demonstrated in serum and plasma, but it is believed that they are of little significance in the mechanism of immunity. It was not possible to demonstrate bactericidal action in fixed tissue cells. The mechanism which produces immunity in tuberculosis is probably due to a physicochemic alteration in the cells by which they become less susceptible to tubercle formation when brought in contact with tubercle bacilli. The tubercle bacilli, then, remain virulent in the tissues, but they do not produce disease. Owing to intercurrent diseases or other physiologic disturbances the tissue may lose this resistance against tubercle bacilli, and the previously latent tubercle bacilli may produce progressive disease.

MAX PINNER.

CRITICISM OF B. LANGE'S STUDIES CONCERNING THE CAUSES OF THE LÜBECK DISASTER. R. KRAUS, *Ztschr. f. Tuberk.* **61**:113, 1931.

It is pointed out that Lange's statement that BCG could not revert to a pathogenic type is unwarranted. Various workers have shown that such reversion does occur, particularly on egg mediums which were used for cultivation in Lübeck. It seems unjustified to conclude that the pathogenic strains that were isolated from dead children were identical with the pathogenic human strain that was kept in Deycke's laboratory at the time of the vaccinations.

MAX PINNER.

## Tumors

PURIFIED (PROTEIN-FREE) VIRUS OF CHICKEN TUMOR NO. 1. MARGARET REED LEWIS and WILLIAM MENDELSON, *Am. J. Hyg.* **13**:639, 1931.

Whether the tumor-producing agent is of the nature of a protein cannot be discussed at present. It can be said, however, that no protein detectable by the tests used remained in the solutions of the tumor virus purified by this method. The surmise is that the behavior of the protein-free virus solutions may prove to be different from that of the unpurified tumor extracts. It is hoped that the study of the protein-free virus may lead to a clearer conception of the nature of the virus and, through this, to a better understanding of how the growth of a tumor is brought about.

AUTHORS' SUMMARY.

INTRAVASCULAR HEMANGIO-ENDOTHELIOMA OF THE OVARY. F. W. SOVAK and V. CARABBA, *Am. J. Obst. & Gynec.* **21**:544, 1931.

Although fifty-two cases of ovarian endothelioma have been reported in the literature, this case is only the fifth of its particular type. A colored woman, 35 years of age, suffered from acute abdominal pain and had a gradually increasing mass in the lower right quadrant of the abdomen. Operation revealed a cystic, lobulated mass, with blood-filled spaces of varying size. Microscopic examination showed a complex tumor, the cellular unit being a small, rounded or flattened, oat-shaped cells. Among the tumor cells were thin-walled blood vessels, evidently derived from the cells. In other areas the cells formed spaces widely dilated with blood.

C. G. WARNER.

TUMORS OF THE CAROTID BODY. F. W. RANKIN and W. L. A. WELLBROCK, *Ann. Surg.* **93**:801, 1931.

The carotid body is a triangular collection of cells connected by fine fibrous strands, with the oral epithelium on one end and the thymus on the other. Embryologically, it is supposedly developed from the epithelium of the pharynx, from the walls of blood vessels and from nerve tissue or from sympathetic ganglion cells of the carotid plexus. Physiologically, little is known about it. Tumors of this body have been designated by such names as adenoma, endothelioma, perithelioma, paraganglioma and simply tumor of the carotid body. The authors give a comprehensive review of the literature and add twelve new cases of carotid tumor, one bilateral. The characteristic structure of these tumors of the carotid body is that of alveolar perithelioma, with whorls of polyhedral and granular cells, or compact groups with or without lumen, surrounded by endothelioma. The tumor is usually vascular, with the blood spaces lined by prominent endothelial cells. About 80 per cent are benign, rarely metastasizing, occasionally recurring. In one case there were metastases in the liver. Of the twelve cases reported by the authors, five were malignant and six were benign; the bilateral case is interpreted as malignant on one side and benign on the other. The criteria for malignancy are: variation in the size of cells; hyperchromatic nucleoli; mitosis; invasion of the capsule by the tumor. Whether benign or malignant, the tumor is slow in growth.

C. G. WARNER.

SACRAL CHORDOMA. J. A. DICKSON and C. A. LAMB, *Ann. Surg.* **93**:857, 1931.

Chordoma is a tumor arising from the cellular remains of the notochord and occurring, therefore, along the spine, most frequently at its extremities. The average age of onset is from 35 to 40, the spheno-occipital chordomas appearing, on the average, ten years later than the sacrococcygeal. The tumor is twice as frequent in males as in females. Chordoma has been produced experimentally in rabbits by puncturing the body of a vertebra. Grossly, the tumor appears well encapsulated, rounded or lobulated, with mucoid degeneration in the lobulated cellular areas. Frequently cells of syncytial type are embedded in mucin, some areas resembling colloid carcinoma. Rarely is mitosis present, and only occasionally metastasis. The case reported in this article was treated after operation with deep x-rays, with a progressive decrease in size and a gradual hardening and calcification.

C. G. WARNER.

MENINGEAL FIBROBLASTOMA (DURAL ENDOTHELIOMA, MENINGIOMA, ARACHNOID FIBROBLASTOMA). CHARLES A. ELSBERG, *Bull. Neurol. Inst., New York* **1**:3, 1931.

Meningeal tumors are supposed to grow from arachnoid cells normally present in the form of clusters or nests in the tissue spaces of the dura mater. The study of tumors of the spinal meninges makes such a postulate, according to Elsberg, not altogether tenable, for such tumors arise also outside the spinal



dura, or on its inner surface, without demonstrable connection with the arachnoid tunic. Elsberg suggests that the origin of the tumors is to be sought in misplaced mesenchymal cell rests. Of the several names suggested, he prefers that of fibroblastoma. The present contribution is based on a study of 100 cases—50 of cranial and 50 of spinal tumors. They are usually slowly growing, affording the brain an opportunity to adjust itself to the changed conditions. Elsberg differentiates three types: hard tumors, well encapsulated, causing atrophy of the convolutions by pressure; soft tumors, which possess a thin limiting capsule, and which for this reason may invade the sulci and separate the convolutions—the tissue of the soft tumors is fragile and usually very vascular—and tumors combining the features of these two; that is, several well encapsulated tumors connected by soft masses. The intracranial pressure, though increased in the presence of these tumors, is usually not high. It is much less than with the subcortical infiltrating tumors. In experienced hands, if the operation is well planned, the removal of a meningeal tumor is followed by brilliant results.

GEORGE B. HASSIN.

MOUSE LEUKEMIA. E. C. MACDOWELL and MAURICE N. RICHTER, *J. Cancer Research* **14**:434, 1930.

Susceptibility to inoculated leukemia is a dominant character in mice; in the backcross to a resistant strain there is a segregation of susceptibility and resistance. Two different lines of inoculated leukemia give different proportions of susceptible mice in the backcross. With the inoculated leukemia from line I, the proportion of susceptible mice is consistently lower than is expected for a single gene, and considerably higher than is expected for two or more independent genes. The hypothesis that there are two linked genes is being tested. With the inoculated leukemia from line A the proportion of susceptible mice in the backcross is always less than when line I is used. But changes in line A itself result in different proportions in different periods; this necessitates interpreting the different periods independently, but such interpretations are not amenable to satisfactory genetic verification.

AUTHORS' SUMMARY.

AN HISTOLOGICAL STUDY OF SALIVARY GLAND TUMORS. A. A. THIBAudeau and E. M. BURKE, *J. Cancer Research* **14**:440, 1930.

In salivary tumors the histologic picture in no way aids in the determination of the relative malignancy or of the outcome. The outcome seems to depend on the clinical aspects of the case, with special reference to the extent of the local infiltration and the duration of the tumor.

B. M. FRIED.

TUMORS IN CAPTIVE PRIMATES (GIANT CELL TUMOR IN A CHACMA BABOON). HERBERT L. RATCLIFFE, *J. Cancer Research* **14**:453, 1930.

The case of a malignant tumor in a primate in captivity which is described in this article, is the seventh on record. The growth originated near the lower epiphysis of the ulna, perforated the shaft and the articular cartilage and infiltrated the surrounding tissue. It grew rapidly, metastasizing to the lungs, the heart and the gluteus muscle. The tumor was made up of large spindle cells, numerous capillaries and giant cells. The author did not succeed in transferring the neoplasm to *Macacus rhesus*.

B. M. FIELD.

PRIMARY MALIGNANT TUMOR OF THE URETER. M. J. RENNER, *Surg., Gynec. & Obst.* **52**:793, 1931.

The author reports what he considers to be a carcinosarcoma of the ureter. This tumor was found in a man 71 years old. The growth originated from

approximately the middle half of the posterior wall of the right ureter, filling the ureter and penetrating into the bladder. The mass in the bladder measured 7 by 4 by 3.5 cm. Histologically, the growth consisted of carcinomatous islands surrounded by myxosarcomatous and cartilaginous tissue.

C. G. WARNER.

LYMPHOSARCOMA OF THE NECK. JOHN W. SPIES, Surg., Gynec. & Obst. **52**: 815, 1931.

Attention is called to certain types of metastatic carcinoma that may readily be confused with lymphosarcoma of the cervical lymph nodes. In a series of fifty cases diagnosed as lymphosarcoma of the neck, five cases of metastatic carcinoma were found, three of which were classed as lympho-epithelioma; the other two were classed as transitional cell carcinoma. These tumors are radiosensitive. Their primary sites are in the nose, pharynx and mouth.

C. G. WARNER.

ALTERATION OF MALIGNANCY IN METASTATIC GROWTHS AFTER REMOVAL OR IRRADIATION OF PRIMARY GROWTHS. RALPH G. MILLS, ALBERT C. BRODERS and HAROLD D. CAYLOR, Surg., Gynec. & Obst. **52**:824, 1931.

The system of grading carcinomas devised by Broders has been applied to the study of a series of fatal cases, to detect, if possible, the potency of various factors that may affect metastatic growths and even the viability of carcinomatous masses.

Alterations in the histologic structure of malignant metastatic growths might be expected to be found, if longevity after operation or after treatment by irradiation is dependent on changes in the cell.

Evidence was not found that removal of the primary growth or irradiation altered, to an appreciable degree, the histologic structure of metastatic malignant growths.

Irreconcilable variation between the grade of the primary malignant tumor and that of the metastatic growths, between different portions of the same primary growth, or between different metastatic growths of the same subject occurred in 32 of 207 cases (the entire series of cases was 225, in 50 of which the variation persisted after the second survey; 18 of the 50 were finally excluded from the series because the question of multiple primary tumor entered in). The cause of this variation seemed to be inherent in the carcinoma rather than connected with the etiologic factors that surrounded it. Growth of metastatic masses in the lung seemed to be slightly inhibited by ecologic factors in the lung, but there was no noticeable influence exerted by any other organ on metastatic growths that developed within it.

A method of approach has been developed that should be applied to a much larger series of cases, in order properly to analyze the factors involved.

AUTHORS' SUMMARY.

INCIDENCE OF CANCER OF THE BLADDER AND PROSTATE IN CERTAIN OCCUPATIONS. S. A. HENRY, N. M. KENNAWAY and E. L. KENNAWAY, J. Hyg. **31**:125, 1931.

In persons pursuing eight of ten occupations associated with exposure to coal gas, tar, pitch or soot, the incidence of cancer of the bladder is greater than in the general male population, and in persons following five of the ten occupations it is from one and a half to four times as great. Three of these occupations show the highest figures for incidence of cancer of the bladder observed among the forty-six occupations investigated. Various possible sources of error in these figures are discussed, of which the most serious is the smallness of the numbers of deaths involved. The corresponding data for cancer of the prostate give less consistent indications of an occupational liability. The subject needs much further inquiry,

but it has seemed best to publish now, without further delay, such material as is available, rather than wait for another long term of years while more data accumulate.

AUTHORS' SUMMARY.

### Medicolegal Pathology

FATAL POISONING WITH ARECALINUM HYDROBROMICUM. HEINSEN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17:67**, 1931.

Arecalin is an alkaloid of *Areca catechu*. Poisonings with this drug were unrecorded in the literature to date. Its pharmacodynamic action is similar to that of pilocarpine. In the case described, the autopsy disclosed marked edema of the lungs and large quantities of mucous secretion and foam in the respiratory tubes. There was arterial hyperemia of all the internal organs, with definite signs of internal asphyxiation of the tissues. This case teaches that 50 mg. of arecalinum hydrobromicum causes the death of a healthy adult man within from seven to twelve hours. The poisonous action of this drug is greater and more intense than that of pilocarpine hydrochloride.

E. L. MILOSLAVICH.

DETERMINATION OF ALCOHOL IN BLOOD AND BRAIN. H. KLAUER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17:89**, 1931.

All the present methods of determining alcohol, quantitatively or qualitatively, are of little value to forensic medicine, because they are either inaccurate or too complicated. To date, the most favored methods used are that of Nicloux, oxidation of alcohol by potassium bichromate in a sulphuric acid solution, and the interferometric procedure of Kionka and Hirsch; but even these are not satisfactory. A new technical method is introduced by the author and should be consulted in the original paper.

E. L. MILOSLAVICH.

SPATTERING OF TISSUE FROM GUNSHOT WOUNDS. WALDEMAR WEIMANN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17:92**, 1931.

Not uncommonly, blood, particles of muscle or of fat tissue or small fragments of fractured bone may be hurled out of a gunshot wound and then be found, even after a long period of time, on various objects in the environment of the victim or on the clothing or body of the assailant. Spattering of such tissues is seen particularly on the weapon itself, and then not only on its outer surface, but also within the barrel. Of course, such particles may lodge on the hand that pulled the trigger. Injuries to the fingers or smudging of the same are occasionally observed on the hand that has fired a pistol, as, for example, in a suicide. Spattering of torn tissues of a gunshot wound is seen under certain circumstances, such as in the use of firearms of large caliber, in instances of the abundant development of explosive gases that penetrate the wound canal, and in cases in which the bullet strikes a bone that is close to the skin, thus hindering the expansion of explosive gases. In the last instance, the gunshot wound exhibits a star-shaped appearance. In wounds of this character, one should search for spatters of tissue. In gunshot injuries of larger blood vessels or of the eyeball, spattering of blood or of crushed tissues of the eye may be pronounced, on account of the hydrodynamic action of the fluid medium.

E. L. MILOSLAVICH.

NECROSIS OF LIVER FROM INDIRECT INJURY. J. GERBER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17:106**, 1931.

Approximately one third of all blunt injuries to the abdomen cause injuries to the liver, particularly to the convexity of the right lobe. The author describes the development of necrotic areas in the subcapsular region on the convexity of

the liver in a young pregnant woman who died of complications (hemothorax) following gunshot wounds of the right side of the thorax. As predisposing factors for the indirect transmission of the force to the liver are mentioned the gravidity hyperemia of the liver, its high position and the limited expansion of the diaphragm, owing to the enlarged gravid uterus. There is a certain analogy to the injuries of the internal organs occasionally found in tangential gunshot wounds of the wall of the thoracic and abdominal cavities.

E. L. MILOSLAVICH.

CHRONIC INDUSTRIAL RADIUM POISONING IN JOACHIMSTHAL. A. WOLDRICH, *Med. Klin.* **27**:17, 1931.

A special investigation has revealed that many workers in the Joachimsthal mines suffer from a secondary myelotoxic anemia, undoubtedly due to the radium in the minerals.

TRAUMATIC HEMORRHAGE IN THE WALLS OF THE FOURTH VENTRICLE. O. BERNER, *Norsk mag. f. lægevidensk.* **91**:1155, 1930.

Berner claims that slight trauma may cause hemorrhages in the region around the fourth ventricle, and that even the changes in tension originating during spontaneous bleeding are under certain conditions sufficient to cause analogous hemorrhages. At the time of the trauma, there occurs in the fluids in the lateral ventricles a wave movement, which, seeking to escape, transplants itself through the aqueduct of Sylvius and strikes the floor of the fourth ventricle with such force as to cause lesions there (Duret). The phenomena from the circulatory organs and from the respiratory side so characteristic in the picture of concussion of the brain are thus, he says, easily explained by hemorrhages in the region where the centers of these functions are situated. He discusses where in the brain spontaneous bleedings are known to occur, with particular attention to the so-called "primary ventricular" hemorrhages which originate from the choroid plexus or from a subependymal blood vessel and thus may enter the cranial cavity without destruction of nervous tissue, and treats of the probability of a spontaneous origin of a hemorrhage in the fourth ventricle.

POSTTRAUMATIC TARDY HEMORRHAGES OF THE BRAIN. FRANCIS HARBITZ, *Norsk mag. f. lægevidensk.* **92**:501, 1931.

Harbitz says that practical experience shows multiple hemorrhages and contusions at the base of the fourth ventricle to be unusual even after marked traumas of the head with pronounced changes in the cranium and surface of the brain; he has sometimes, though seldom, found hemorrhages in the inner portion of the brain simultaneously with contusion of the cortex, meningeal bleeding and fissures in the cranium, as after automobile accidents, but never certain or highly probable traumatic hemorrhages in the walls of the third and fourth ventricles as the only essential result after a moderate trauma of the head, with concussion of the brain as the cause of death (see Berner: *Norsk mag. f. lægevidensk.* **91**:1155, 1930), and he regard such results as rare and hardly of practical significance. In his opinion, Berner stresses too much these small traumatic hemorrhages in the fourth ventricle as the cause of death when there are other evident changes in the brain that can both explain the symptoms and be the cause of death, and in cases with intoxication the possibility cannot be excluded that the changes and death might be due to alcoholism. He agrees with Berner that in concussion of the brain real anatomic changes are doubtless far more frequent than supposed, and more attention to such changes in the walls of the third and fourth ventricles is now advised. With regard to tardy hemorrhages on a traumatic basis, in his material only two cases to date have revealed what might plausibly be considered as late hemorrhages of traumatic origin, and they occurred in work worn men, aged 42 and 55, respectively.

## Technical

A NEW METHOD FOR THE DETERMINATION OF CHOLESTEROL. A. BLOCH, Schweiz. med. Wchnschr. **61**:108, 1931.

The method originally proposed by Bloor (*J. Biol. Chem.* **24**:227, 1916) for the colorimetric determination of cholesterol in blood, while widely used for many years as a standard procedure, was not universally accepted, because of the time required to make the test, the volume of the reagents required and the frequency with which an undesirable brown contamination would appear during the final stage of the development of color. Apparently unaware of the several existing modifications of the original Bloor method that have appeared within recent years, notably that of Sackett (*J. Biol. Chem.* **64**:203, 1925), which requires only 0.2 cc. of blood, is far simpler and more rapid and yields an excellent preparation for colorimetry, Bloch proposes an additional modification that requires the preliminary separation of the cholesterol on precipitated barium carbonate. One cubic centimeter of the serum is heated on a water bath for one hour with 20 cc. of 10 per cent barium hydroxide. After cooling and filtration, the precipitated material is allowed to dry. The cholesterol is then removed from the precipitate by prolonged extraction with a mixture of alcohol and ether. An aliquot volume of the extract is evaporated to dryness and the chloroform-soluble fraction of the residue converted into the familiar green end-product of the Liebermann-Burchard procedure. Precipitation with the Folin-Wu deproteinization reagent may be substituted for precipitation with barium hydroxide.

ARTHUR LOCKE.

SMEAR PREPARATIONS IN LYMPHATIC LEUKEMIA. K. A. HEIBERG, *Centralbl. f. allg. Path. u. path. Anat.* **50**:101, 1930.

From studies on the blood of six normal people and three suffering from chronic lymphatic leukemia the author concludes that the nuclei of only 10 per cent of the small lymphocytes of normal people are greater in diameter than 10 microns, whereas from 37 to 50 per cent of the lymphocytes of those ill with leukemia are larger than 10 microns. He asserts that distortions of as much as 150 per cent occur in the cells of sections. He thinks that smear preparations carefully made yielded the more accurate results.

GEORGE RUKSTINAT.

A METHOD FOR INVESTIGATING PATHOLOGIC ALTERATIONS IN BONE STRUCTURE. L. S. FRANK-KAMENTZKY and W. J. SCHLAPOHERSKY, *Centralbl. f. allg. Path. u. path. Anat.* **50**:133, 1930.

The authors employed roentgenograms of thin sections of bone in their studies. Photographs of the changes noted in rickets, osteitis deformans and chronic osteomyelitis and of changes observed post mortem illustrate the potentialities of the method first employed by Julius Wolff in 1900.

GEORGE RUKSTINAT.

FROZEN UNFIXED TISSUE SECTIONS FOR HISTOLOGIC STUDY. O. SCHULTZ-BRAUNS, *Centralbl. f. allg. Path. u. path. Anat.* **50**:273, 1931.

Unfixed tissues were cut on the freezing microtome with the added agency of a second freezing apparatus that cooled the knife. From the knife the sections, still frozen, were transferred to slides. In this way friable material, such as placenta, and sticky substances, such as mucus and fat, can be cut and the sections handled with ease. A second advantage of the method is that sections are placed on the slides in the original state without displacement or partial solution of any of the constituents. After being transferred to a slide, the sections can be fixed quickly and individually. The method eliminates fixation of numerous blocks and guarantees complete fixation, which is hard to achieve in the center of a block.

GEORGE RUKSTINAT.

A MODIFICATION OF CAJAL'S GOLD SUBLIMATE METHOD FOR DEMONSTRATING MACROGLIA CELLS IN FORMALDEHYDE-FIXED MATERIAL. M. CORTEN, *Centralbl. f. allg. Path. u. path. Anat.* **50**:339, 1931.

The steps in this staining method are as follows:

1. Cut frozen sections of formaldehyde-fixed material, not thicker than 25 microns.
2. Immerse in the following mixture: ammonium bromate, 15; formaldehyde (neutral), 100, and distilled water, 400. Warm until the mixture steams.
3. Transfer without washing to: antiformin, 3 cc.; distilled water, 2 cc., and alcohol (96 per cent), 8 cc. Keep the sections in motion in this solution for from six to fifteen seconds.
4. Wash twice quickly in distilled water and then place in 1 per cent aqueous gold chloride, 4 cc.; 5 per cent aqueous silver nitrate, 8 cc., and distilled water, 6 cc. Keep in the dark at 37 C. for one hour or until adequately impregnated.
5. Wash in 5 per cent sodium thiosulphate for fifteen minutes, wash in water, dehydrate, clear in xylene and mount in balsam.

The results are similar to those obtained by the original method of Cajal. The modification is applicable to gelatin sections, if the concentrations of gold chloride and sublimate are slightly altered.

GEORGE RUKSTINAT.

DEATH AFTER TRANSFUSION OF BLOOD. H. WILDEGANS, *Deutsche med. Wchnschr.* **56**:2031, 1930.

Severe reactions follow about 5 per cent of transfusions of blood regardless of whether typing is done or not. Errors in typing may occur because the serum is exposed to sunlight or because of alkali in the test tubes. Theoretically a transfusion can be undertaken without accident if the red blood cells of the donor are not agglutinated by the serum of the recipient. Other factors must, however, be considered. In some cases, the blood of the recipient is in such a small volume that it cannot effectively dilute the blood of the donor. For this reason transfusions should never be given in quantities greater than one fifth of the donor's total circulating blood. Wildegans advises administering a saline or a dextrose solution as a preliminary procedure and then calculating, from the difference in the reading of hemoglobin before and after this transfusion, the volume of the blood of the recipient. Agglutination has been observed to occur in titers of 1:250. An important feature in death occurring shortly after a transfusion is the presence of hemoglobin in the kidney, as emboli in the glomeruli and as casts in the tubules. In biologic processes hemolysis can occur in the absence of agglutination, and this suggests a simple hemolysis test to make the transfusion of blood safer.

GEORGE RUKSTINAT.

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, April 23, 1931*

LEILA CHARLTON KNOX, *President, in the Chair*

TWO UNUSUAL CASES OF INTERSTITIAL MYOCARDITIS. MAX LEDERER.

CASE 1.—The patient, a boy, was born at full term but was delivered by forceps, and was formulae-fed. At 7 months of age he began to have diarrhea, which was controlled by diet. At 9 months, he had another attack of diarrhea, with from four to eleven loose, watery stools, showing mucus, but no blood which had lasted for ten days when he was admitted to the hospital. In these ten days he had lost 3 pounds (1.4 Kg.).

He was very poorly nourished, dehydrated and apathetic. The breathing was rapid and shallow, the eyes sunken and the fontanels depressed; there was no cyanosis. The extremities were normal. There was an inconstant, but marked, lateral strabismus of the right eye. The tongue was dry; the tonsils were normal; the cervical and inguinal lymph glands were palpable. The chest was poorly developed; the lungs were normal; the intercostal spaces were prominent. The following observations were made regarding the heart: The point of maximum impulse was in the fifth intercostal space in the nipple line. On percussion there was no enlargement. On auscultation the sounds were rapid and of poor quality; there were no murmurs. The second pulmonic sound was louder than the second aortic.

The urine showed 1.4 per cent sugar, a faint trace of albumin and a trace of acetone. On subsequent examination the urine was clear. The hemoglobin was 82 per cent; the red blood cells numbered 4,500,000; the white blood cells, 18,000, with lymphocytes 81 per cent. The white count varied between 6,100 and 18,000, with from 18 to 57 per cent polymorphonuclears. Nose and throat cultures were negative for diphtheria bacilli. The tuberculin reaction was negative. Stool cultures showed colon bacilli. Repeated roentgenograms of the chest were negative.

Because of the strabismus a spinal puncture was done, and the fluid was found to be normal. The child was placed on an appropriate diet, given two transfusions and within seven weeks was discharged, apparently normal.

The diagnosis on discharge was diarrhea with intoxication and acute catarrhal otitis media.

The child was readmitted nearly one year later. During the interval he had had two mild attacks of tonsillitis, and two months before readmission diarrhea, with twelve stools per day. Two months before admission he had been fretful and had had a temperature of from 101 to 104 F., without chills. Ten days before admission his gums had become swollen and covered with yellow pus. Blisters appeared on the tongue. There was no bleeding. He was fretful and did not eat. The stomatitis was treated, without improvement.

The pertinent findings were swelling and redness of the left eyelid, with a thick, yellow, purulent discharge, and injection of the conjunctivae. The right eye was normal. From the mouth emanated a fetid odor. The tongue was coated and denuded in small areas. The gums were swollen and bled easily. There were no ulcerations. The pharynx was red. The tonsils were large and cryptic. The heart showed no enlargement. The point of maximum impulse was in the fifth intercostal space, just within the nipple line. There were no thrills or murmurs.

The sounds were rapid, irregular and almost tick-tack in quality. There was a suggestive blowing quality to the first apical sound. The second apical sound was accentuated. The pulmonic second sound was accentuated and louder than the second aortic. There were a few discrete, enlarged lymph nodes, in the cervical, axillary and inguinal regions.

The urine showed a faint trace of albumin and acetone. The hemoglobin ranged between 64 and 51 per cent; the red blood cells, between 3,100,000 and 3,450,000; the white cells, between 20,200 and 9,000, with 68 to 59 per cent polymorphonuclears. The bleeding time was one and a half minutes; the coagulation time, three minutes. The tuberculin test was negative up to 5 mg. Cultures from the nose and throat showed the usual organisms; those from the mouth showed a predominance of streptococci. The temperature ranged between 99.4 and 104 F., in a septic fashion. The pulse rate fluctuated between 110 and 132 and the respirations between 28 and 60.

Shortly after admission signs of bronchopneumonia developed, and death followed on the eleventh day.

*Autopsy.*—The body was that of a well developed, well nourished white boy, 21 months old. The lower extremities were slightly edematous. The abdominal cavity contained between 1 and 2 liters of pale yellow, clear fluid. The peritoneum was pale, smooth and glistening. Both pleural cavities contained small amounts of a similar fluid. The pericardial sac was filled with a similar fluid, causing moderate distention. The thymus and the lungs were edematous, the lungs containing a moderate number of pigment macrophages.

The heart was enlarged, weighing 100 Gm. Externally the right ventricle was distorted by a smooth, round mass that occupied the upper anterior aspect of the right ventricle, close to the interventricular septum. It was nodular and firm. When sectioned, this area was found to consist of a rather well circumscribed, homogeneous, firm, but not hard, yellow tissue embedded in the wall of the myocardium, displacing the epicardium outward and the endocardium inward. The nodule measured 2 by 3 cm., and extended upward to the base of the left cusp of the pulmonary valve, without involving it or impairing its efficiency. There was a similar nodule of the same size embedded in the anterior wall of the right ventricle near the apex. At this point the endocardium and papillary muscles appeared as white structures. The myocardium as a whole was pale. The foramen ovale and the ductus arteriosus were closed. The tricuspid, pulmonary, mitral and aortic valves were normal. The pulmonary artery, aorta and coronary vessels, as well as the auricles, appeared normal.

Numerous sections were taken from various parts of the organ and fixed in alcohol, formaldehyde, Zenker's and Helley's fluids. They were stained with hematoxylin-eosin, van Gieson's stain, Mallory's stain and aniline blue for Aschoff bodies, elastic tissue, tubercle bacilli, *Spirochaeta pallida* and other bacteria. A study of the sections revealed what appeared to be a progressive inflammatory process passing through successive acute, subacute and chronic stages, with final replacement of the parenchyma by scar tissue. In the acute lesions was seen an interfibrillary infiltration of the musculature with polymorphonuclear neutrophils, accompanied by edema. The muscle fibers in these areas were thinned, as though compressed, and appeared to be undergoing necrosis. The cells were distorted; striations had disappeared, and in places only fragments of cells could be recognized. In the subacute stage, the muscle elements had almost completely disappeared, having been replaced by large collections of numerous plasma and round cells, a few eosinophilic polymorphonuclears and an occasional giant cell. In the chronic stage, the exudate consisted almost exclusively of small round cells and many fibroblasts, the muscle cells having completely disappeared. The stroma was loose, and fibrous tissue strands could be recognized. In the final stage, all that remained was dense fibrous tissue, containing newly formed blood vessels, a few round cells and no muscle. The endocardium, pericardium and blood vessels were normal.



No Aschoff bodies, tubercle bacillus, *Spirochaeta pallida* or other bacterium could be found.

The mediastinal and mesenteric lymph glands were soft, enlarged and pink. On microscopic examination, the normal architecture was exaggerated. The lymphoid follicles were small, but well developed. The germinal centers showed a variety of changes, ranging from hyperplasia to fibrosis and hyalinization. Some were increased in size owing to proliferation of their component cells; in others, the cells were necrotic. The necrosis varied from that of mild degree to complete destruction of cells and replacement by detritus and nuclear fragments and in some areas fibrosis and hyalinization. The pulp was congested and edematous. The spaces were large and filled with small round cells. In the mesenteric nodes were only moderate numbers of macrophages.

The spleen weighed 50 Gm. It was normal in shape, purple and firm. The capsule was smooth. On section, the follicles stood out prominently. The pulp was dark red and appeared congested.

Microscopically, the architecture of the spleen was normal. In the lymph follicles were changes resembling those in the lymph nodes. In the spleen, however, the changes were more severe, and involved more follicles than in the lymph nodes. The pulp was congested and contained numerous small hemorrhages. The walls of the sinuses were thickened, the thickening being accompanied by marked endothelial hyperplasia.

The capsule of the liver was pale, smooth and glistening. On section, the cut surface presented a widely diffuse, yellow appearance.

Microscopic sections showed the capillaries to be greatly dilated, thereby causing marked distortion of the liver cords. The hepatic cells were swollen in some places and compressed in others. They showed fatty degeneration and infiltration. Areas of focal necrosis, some in early stages, with destruction of the liver cells and invasion with polynuclear cells, others with round cell infiltration and still others with fibrotic changes, were scattered throughout the organ. In the portal areas were an abundant round cell infiltration and a moderate increase in fibrous tissue. The bile passages, except for an apparent increase in the diameter of their lumina, were normal.

The common duct was patent, as were the hepatic ducts. The gallbladder and cystic duct were absent; a thin fibrous band of tissue represented the site where this organ is normally found. The hepatic artery and the portal vein were normal.

The kidneys weighed 60 Gm. each. The capsules stripped with ease, leaving smooth, pale, lobulated surfaces. The cut surfaces of the organs showed the cortex and medulla well differentiated, but pale. The blood vessels were congested. The pelves were normal.

Microscopic sections showed most of the glomeruli to be normal. Scattered throughout the organs, however, were glomeruli that showed changes: some were congested, enlarged and hyperplastic; others showed typical crescent formation and lesions indicating transformation from early crescent formation to complete fibrotic replacement of the tufts. No cellular exudate could be found. The tubular epithelium was swollen and somewhat granular. The blood vessels were normal.

*Anatomic Diagnosis.*—The anatomic diagnosis was: acute, subacute and chronic interstitial myocarditis; congenital absence of the gallbladder and cystic duct; toxic necrosis of the lymphadenoid tissue (lymph node and spleen); subacute and chronic glomerulonephritis; interstitial hepatitis, and chronic passive congestion and focal necrosis of the liver.

**CASE 2.**—A boy, aged 13 years, complained of weakness in the legs, chills and fever, vomiting of four days' duration, difficulty in breathing, bloody stools and hematemesis.

The patient was one of nine children; the others were living and well. He had had pneumonia when he was 6 years old. Two weeks before admission to the hospital on Nov. 30, 1930, during a football game he was hit with the football in the right upper quadrant of the abdomen. The next day, he noticed weakness of the legs. That night he was unable to eat and vomited yellowish fluid. He felt

no pain in the upper right quadrant, but noticed that it was black and blue. The next night he complained of pain in the epigastrium and felt feverish. He went to school until November 24, and then remained at home because of his illness.

On the twenty-sixth he began to have chills, which lasted one-half hour and were followed by fever. The weakness in his legs continued. On the twenty-seventh, he noticed that his stools were watery and black, and he coughed up a few firm, red pieces of blood. On the twenty-ninth, he began to have difficulty in breathing, because of pain. There was no cough or expectoration.

Physical examination showed the boy lying in an orthopneic position, apparently acutely ill, with rapid, shallow respirations and dilatation of the alae nasi. He had a cyanotic flush on his cheeks. The cervical lymph glands were palpable and enlarged. Abnormal venous pulsation was noted in the neck. The thyroid gland was not palpable. The chest was well developed. The respirations were rapid and shallow. There was some dullness at the base of the right lung, where the breath sounds were diminished. The heart sounds were rapid, but regular in rate and rhythm. There was an occasional dropped beat. The sounds were of good quality. There were no murmurs. The heart was not enlarged to percussion. The abdomen showed tenderness and rigidity in the upper right quadrant on deep palpation. The edge of the liver was palpable at the umbilicus.

The temperature on admission was 99 F., and during the stay in the hospital ranged between 99 and 101 F. The pulse rate varied between 96 and 138. The respirations were between 26 and 50.

The blood count on admission showed 2,440,000 red blood cells, 16,200 white blood cells, with 72 per cent polymorphonuclear leukocytes, and hemoglobin, 68 per cent. The blood culture was sterile. The Wassermann and Kahn tests were negative. Blood was present in the stools.

During his stay in the hospital, the patient began to show symptoms and signs of cardiac decompensation, and he died on Dec. 8, 1930.

*Autopsy.*—The body was well developed and well nourished. The peritoneal cavity contained a large quantity of straw-colored, clear fluid. The right pleural cavity was obliterated by dense adhesions; the left contained straw-colored fluid. The pericardial sac contained a slightly increased quantity of fluid.

The heart weighed 212 Gm. The epicardium was smooth and glistening. The musculature was flabby. Both auricles were dilated. The foramen ovale was patent. The mitral, pulmonary, tricuspid and aortic valves were normal. The musculature of the right ventricle measured 4 mm.; that of the left, from 9 to 11 mm. On section there were extensive areas of yellowish-gray, dense tissue scattered throughout both walls. The interventricular septum showed a similar appearance, the upper quarter being very thin and translucent. Large thrombotic masses adhered to the walls of the apexes of both ventricles. The coronary arteries appeared normal.

Microscopic sections showed extensive areas of destruction of cardiac musculature, with replacement by granulation and fibrous tissue, a picture similar to that seen in myomalacia and myofibrosis due to coronary occlusion. The coronary arteries, however, were normal, except that the terminal fine twigs contained organized thrombi. No spirochetes were found after prolonged search.

The other organs showed the usual changes found in chronic passive congestion.

#### DISCUSSION

ALFRED PLAUT: Might not the last case be an interstitial myocarditis? It happens, although very seldom, that a person dies in seemingly perfect health, and the autopsy shows nothing except a certain dilatation of the heart, and unless microscopic examination of the heart muscle is made, the case remains a mystery. The fact that the disease started after the accident with the football perhaps is entirely accidental.

As to the other case, I should like to know how far the bacteriologic examination of the autopsy material has been carried—whether any material was used

for cultures or animal inoculations. Perhaps that might have been one possibility of learning something of this widespread inflammation, and the fact that all the stains for bacteria were negative would possibly not exclude an infection with the pseudotuberculosis group, which are very difficult or impossible to stain in sections.

COLEMAN RABIN: The first case reminds me of a Negro woman on whom an autopsy was performed at the Mount Sinai Hospital. She had recently come from the West Indies, and entered the hospital with cardiac failure, dying soon thereafter of what appeared to be acute myocardial failure. At the postmortem examination there was found a dilated heart, which showed necrosis of muscle fibers, old fibrous tissue and fresh granulation tissue—a picture quite similar to the one shown in the slide. The clinical story was the same—acute cardiac failure in a patient who had apparently been perfectly well, and who showed at postmortem examination an old cardiac lesion of the type described by Dr. Lederer. Similar cases have been described of acute cardiac failure in beriberi; in these cases, coolies drop dead in their tracks, and post mortem an old lesion of the heart is found—a clinical and postmortem picture altogether similar to that in Dr. Lederer's case. I may suggest that although this is probably not beriberi, the diarrhea and hemorrhages are suggestive of a nutritional disturbance.

MAX LEDERER (closing the discussion): The diagnosis of interstitial myocarditis is very tempting in both of these cases. I think when one looks up the question of interstitial myocarditis, one usually finds that there is some etiologic factor to which the lesion can be traced. Those cases in which the myocardium alone is affected without known etiology and without other changes in the body are known as isolated myocarditis. The second case may possibly belong to that group. This boy was 13 years of age. He had been perfectly well until he was hit with the football. He was a big, strapping boy for his age. He had had pneumonia at 6, and he had evidences of old pleurisy. The lung was adherent, and there was no fluid on that side, whereas there was some on the other side. I thought that he had a myocardial condition that began at that time and continued until his heart failed. There was no acute lesion. The question of nutritional disturbance I think may be ruled out.

In the first case there was a question of nutritional disturbance for a long time, with the continued diarrhea. We did not make postmortem cultures or any experiment on animals. In our hospital the results with postmortem cultures are discouraging, because our material comes to us so long after death.

The question of beriberi was not gone into.

The strange thing is that the conditions in these hearts occurred in persons at such a young age: 21 months and 13 years. As I said in my opening remarks, I presented them mainly because of the fact that pathologists who had seen a great many hearts told me they had never seen any just like these.

#### A CASE OF ENDOCARDITIS DUE TO BACTERIUM ACIDI-LACTICI. LEWIS DICKAR.

This appears to be the first case of bacterial endocarditis due to *B. acidi-lactici* reported in the literature.

The micro-organism is considered to be a separate species in the *B. coli* group; others regard it as one of the varieties of *B. coli*. It resembles the other bacilli in this group, but is readily distinguished from them by its lack of power to ferment saccharose and salicin.

One other example of infection with *B. acidi-lactici* was reported by Ray in 1923—a case of meningitis in which the micro-organism was isolated from the blood and spinal fluid. Several cases of endocarditis due to *B. coli* are known.

*History.*—A man complained of frequency of micturition and nocturia. The only previous illness of interest was a mastoiditis with operation two and one-half years before. His present illness began abruptly two days before admission, with marked frequency of urination, nocturia and fever. The temperature was 102 F.; the pulse rate, 96; the respiration rate, 20; the blood pressure, 144 systolic and

60 diastolic. He was an obese man of 54, acutely ill. The chest and abdomen appeared to be normal. The heart sounds were of fair quality, and no murmurs were heard. The prostate was prominent, soft and tender. There was slight hematuria after the rectal examination.

The red blood count was 3,700,000 on admission and dropped to 3,000,000 shortly before death. The white count was 10,000 on admission. The urine showed the presence of albumin and diacetic acid on one occasion. White blood cells were always found.

The temperature rose to 106 F. on November 10. On November 15, an external urethrotomy with incision and drainage of a prostatic abscess was performed. Several drachms of pus was removed. No culture was taken. A blood culture on November 17 was positive, a gram-negative bacillus being isolated. The white count rose to 27,850, with 92 per cent polymorphonuclear leukocytes. A blood culture on November 26 was sterile. The temperature began to drop slowly until November 29, when it became septic. The white count at this time was 15,000, with 91 per cent polymorphonuclears. A blood culture on December 2 was positive. On December 3, a physical examination revealed dullness at the base of the right lung and râles at the base of the left lung. On December 4, bronchovesicular breathing was present on the left side. A loud systolic and a soft diastolic murmur were heard for the first time. These became louder from day to day. The pulmonary signs continued changing. On December 7, petechiae were found in the left conjunctiva, and the fingers on the right hand were painful. Petechiae appeared elsewhere; in addition, the right wrist became painful and swollen. On December 10, the patient was slightly irrational. The white count had again risen to 26,400, with 89 per cent polymorphonuclears. On December 11, the patient was exceedingly irrational; meningismus developed in the afternoon. That evening he suddenly became livid, and the pulse rate dropped to 48. He died thirty-five days after admission and twenty-seven days after operation.

The clinical diagnosis was subacute bacterial endocarditis due to *B. acidilactici* and abscess of the prostate.

*Autopsy.*—The autopsy was performed nine and one-half hours post mortem. There were many petechiae in both conjunctivae and in the skin of the trunk and upper extremities. The perineal incision was clean and not draining. The most striking findings were in the heart.

The heart weighed 500 Gm. The auricles were covered with thick, gray, firmly adherent membrane. Otherwise the right auricle was normal. The tricuspid valve was thin and delicate. At the base of the septal leaflet was a nodule about 0.5 cm. in diameter. On section an abscess was found, surrounded by a hemorrhagic area. The right ventricle was dilated and hypertrophied. The pulmonic valve was normal. In the left auricle was an elevated hemorrhagic area in the fossa ovalis. The mitral valve was slightly thickened at the line of closure. Several small hemorrhagic areas were present in the base of the valve. A hemorrhagic area about 3 mm. in diameter was present in the posterior papillary muscle. The left ventricle was hypertrophied and dilated. The aortic valve leaflets were covered with vegetations, which were soft, crumbly and pale yellow. The largest vegetation measured 2.5 cm. in length and 1.5 cm. in thickness, and entirely covered the right posterior leaflet. This leaflet was ulcerated, leaving an opening about 2 mm. in diameter. Extending from this were small masses, growing down over the endocardium of the left ventricle, the aortic leaflet of the mitral valve and to the base of the tricuspid valve through the muscle. The areas to which the vegetations were adherent were hemorrhagic. The myocardium was firm, with no visible fibrosis.

The coronary arteries were mildly sclerotic. The aorta had many small atheromatous plaques.

The lungs were voluminous and crepitant throughout. The spleen weighed 280 Gm. It was soft and reddish brown. On section the pulp bulged over the capsule. The corpuscles were large.

The liver weighed 3,320 Gm. and was normal.

The gallbladder was large and filled with thick, greenish-black bile. Two stones were present. The wall of the gallbladder was thickened.

The pancreas and the suprarenal glands were normal.

The kidneys each weighed 260 Gm. The capsule of the right kidney stripped easily, leaving a very finely granular surface, which was pale yellowish green with many small light yellow areas. On section the color was yellowish brown. The cortex was 8 mm. in thickness. Small abscess cavities filled with yellowish pus were present. Many tubules in the pyramids were seen as yellowish stripes. The left kidney had a more granular surface. It was yellowish brown, with small elevated yellowish areas. On section it was pale reddish brown. The cortex was thin, measuring 5 mm. in thickness. Red longitudinal stripings were present. The pyramids were made up of alternate stripes of red and yellow. In them were several large abscess cavities up to 1.5 cm. in diameter, filled with pus.

The bladder was thick-walled. The mucous membrane was pale.

The prostate was not enlarged. It was firm and asymmetric. The left side was larger than the right. The capsule was indistinct and merged with the periprostatic tissue. Many small pus-filled abscesses were present. The seminal vesicles and testes showed no changes.

The gastro-intestinal tract was normal.

The brain weighed 1,450 Gm. Externally, there was a hemorrhagic area on the parietal lobe of the right cerebral hemisphere close to the midline. On section many small pale red areas were found scattered through the cerebral hemispheres, internal capsules, cerebellar hemispheres and medulla. The spinal cord was not removed.

*B. acidi-lactici* was obtained in pure culture from the cardiac blood.

*Microscopic Examination.*—In the myocardium were areas of polymorphonuclear infiltration. Plugs made up of short fat rods filled many of the blood vessels. A section of the myocardium taken from a point near the aortic valve showed thickening of the endocardium and subendocardial layers. Parts of these structures were infiltrated with red blood cells and polymorphonuclear leukocytes, and then were replaced by a mass of fibrin containing many polymorphonuclear leukocytes, bacilli and necrotic debris. The necrosis extended into the myocardium with the formation of an abscess. The muscle fibers around this area were necrotic and disintegrated. A methylene blue (methylthionine chloride, U. S. P.) section showed the presence of many bacilli.

The entire aortic valve leaflet taken for microscopic study was necrotic. The surface was covered with vegetations containing large masses of bacteria, polymorphonuclear leukocytes and nuclear debris. The micro-organism was found to be a gram-negative bacillus.

The aorta showed the changes of moderate arteriosclerosis.

The sections of the lungs were normal.

The liver was normal in structure. The portal areas were cellular and contained polymorphonuclears. The sinuses were congested. In many areas masses of bacteria were also present. Many polymorphonuclear leukocytes were found, which were most numerous in the periportal fields.

The gallbladder was thick-walled. The submucous layer was congested and infiltrated by plasma cells.

In the pancreas, polymorphonuclear leukocytes were increased in numbers in a few areas where the acini were shrunken or disintegrated.

The lipoid material in the suprarenal gland was diminished. Bacterial plugs were found in several blood vessels.

Several sections of the kidney showed abscess cavities of varying size in the cortex and medulla. Areas of round cell infiltration were present. Bacterial plugs were found in several glomerular tufts.

The acini of the prostate contained desquamated cells and debris. In a few, necrotic material and polymorphonuclear leukocytes were found. The stroma

was infiltrated with round cells. In another section several abscesses were found. Dense round cell and plasma cell infiltrations were present in the stroma. Many bacilli were present in the sections stained with methylene blue.

The tubules of the testes had thickened basement membranes. Several blood vessels were present, with the walls and surrounding tissue infiltrated with polymorphonuclear leukocytes, round cells and plasma cells.

Several sections of the cerebrum were examined. In one localized area, the meninges were infiltrated by many polymorphonuclears and round cells. Bacterial plugs occluded several blood vessels in the substance of the brain. About these vessels were polymorphonuclears and round cells, which for the most part were sharply perivascular; in only one area in the cerebrum was the brain itself involved.

*Anatomic Diagnosis.*—The anatomic diagnosis was: chronic and acute prostatitis with abscess; external urethrotomy, with perineal incision and drainage of the prostatic abscess; cystitis cystica; acute bacterial endocarditis (*B. acidi-lactici*) of the aortic valve, with extension to the mitral and tricuspid valves; bacteremia (*B. acidi-lactici*), with metastases in the myocardium, pancreas, liver, kidneys, testes, brain and leptomeninges; acute splenic tumor; chronic cholecystitis, and cholelithiasis.

*Comment.*—The original focus of infection in this case was most probably the prostate gland. The portal of entry in most cases of sepsis due to *B. coli* is the urinary tract. Invasion of the blood follows cystoscopy or surgical trauma. This patient showed no signs of involvement of the heart when he entered the hospital. Following the operation there was invasion of the blood with localization of bacilli on the aortic valve leaflets.

#### DISCUSSION

GEORGE CAHILL (by invitation): This patient had an abscess that localized back of the right side of the prostate. Two years previously he had had a mastoiditis requiring two radical operations within three weeks. I had a suspicion that the prostatic abscess was secondary to the mastoiditis, because two years ago a similar case was seen in which, on opening and draining, Friedländer's pneumobacillus was present in the pus and blood stream, as proved on culture. The patient proved clinically to have a "feeder" in the right jugular vein. At first cultures in the present case showed a gram-negative bacillus with a capsule, and naturally it was suspected of being a pneumobacillus. The bacteriologic department, however, on culture showed that the infecting organism was *B. acidi-lactici*. However, clinically, I still think that the prostatic abscess was metastatic. Although no blood culture was taken before the operation, on reviewing the case, I considered that the patient then must have had bacteria. The abscesses in the kidneys were without any local symptoms. This is a common observation in genito-urinary cases. I hoped that the mastoid might show some infection, because the x-ray film showed some cells still in the mastoid, but clinically it was silent. This, in view of the former case, was an interesting point, because from a history obtained from the family later on, the illness apparently had started several weeks before the patient admitted that he was ill.

#### BARTONELLA MURIS ANEMIA: IV. PATHOLOGIC CHANGES DURING THE ACUTE ANEMIA. DAVID PERLA and (by invitation) J. MARMORSTON-GOTTESMAN.

The pathologic changes that follow a severe infection with *Bartonella muris* and the associated anemia in the adult or young albino rat are: first, the changes that are stimulated by the release of large quantities of cellular débris into the circulating blood, phagocytic activity and hyperplasia of the endothelial elements of the liver, thymus and lymph nodes, and in the young rat, of the spleen, with resultant capillary thromboses and focal necroses; second, those changes resulting from the anemia: fatty metamorphosis of the heart, liver and kidneys. A third element is a severe nephrosis, with, in some instances, a degenerative process in the glomeruli. In the bone marrow, hyperplasia of the erythropoietic elements occurs.

BARTONELLA MURIS ANEMIA: V. COMPENSATORY PHENOMENA FOLLOWING SPLENECTOMY IN THE ADULT ALBINO RAT. J. MARMORSTON-GOTTESMAN (by invitation) and DAVID PERLA.

In *Bartonella muris* carrier stock following splenectomy and recovery from the anemia, certain changes are observed in the lymphoblastic and reticular and endothelial elements of the body. These changes appear from three to five months after splenectomy and are associated with immunity to further infection with *Bartonella muris*. The changes consist primarily of hyperplasia of the hemolymph tissue, hyperplasia of the reticular and endothelial elements of the lymph nodes, the formation of lymphoblastic foci periportally in the liver and peribronchially and perivascularly in the lung, regeneration of all elements of the thymus and marked hyperplasia of all elements of the bone marrow (increased hematopoiesis).

#### COMMENT

MENDEL JACOBI. In the experimental production of amyloid in mice on which we reported at the meeting of another society, the lesions we found preceding the development of adult amyloid resembled almost identically those which Dr. Gottesman presented. I should like to ask if in their rats there was any suggestion, either grossly or histologically, that might lead them to suspect that they had some amyloid.

DAVID PERLA: There was not a trace of amyloid; nothing to suggest it grossly or microscopically.

MENDEL JACOBI: Have you used the congo red specific stain for amyloid?

DAVID PERLA: No, there was no suggestion of amyloid.

MENDEL JACOBI: We have found that, even when there was no gross or microscopic suggestion of amyloid, with the specific stain for it, it could be demonstrated.

DAVID PERLA: Where would you suggest that we look for it ?

MENDEL JACOBI: In the reticulo-endothelial cells.

DAVID PERLA: I personally should not place much confidence in such a finding, and I should be dubious if we could prove by a specific stain that there was amyloid deposited in the cells, when there was no gross or microscopic evidence of it.

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*Regular Meeting, May 28, 1931*

LEILA CHARLTON KNOX, *President, in the Chair*

A CASE OF FOREIGN BODY IN THE ORBIT. REUBEN GOODMAN.

The cases reported in the literature may be divided into two groups: (1) cases of long duration, including (a) those unaccompanied by discomfort or symptoms, (b) those presenting symptoms after a variable period and in which removal of the foreign body gives satisfactory results, and (c) those in which removal is followed by unsatisfactory results; (2) cases of short duration, including (a) those without ill effects and (b) those with ill effects. The following case is presented because of the interesting history and unfortunate outcome.

A boy, aged 2, was admitted, on March 18, 1931, to the Jewish Hospital of Brooklyn with a history of a fever of 104 F., and prostration for two days. The family and past histories were irrelevant. One week previously, the patient accidentally lacerated his left upper eyelid with a sharpened end of a lead pencil. He

was taken to a hospital, where the wound was sutured with one stitch, and he was given two injections of tetanus antitoxin. He was apparently well until three days before admission; then he became irritable and listless. The temperature rose to 102 F. The day before admission, he vomited several times. The vomiting was projectile. He rolled his head and eyes from side to side, resented being touched, and would "shiver" all over, as though convulsed, on being handled. On the morning of admission the child became comatose, and the temperature rose to 105 F.

Physical examination showed the child to be well developed and well nourished, acutely ill and comatose. There was proptosis of the left eye. The jaws were held clenched, but could be opened. The patient ground his teeth frequently and was foaming at the mouth. The skin, lymphatic system and head were normal. Both eyes were somewhat chemotic; the blood vessels were full. Both eyes were fixed, and there was an optic neuritis bilaterally, more marked on the left. The disks were swollen and their outlines blurred. The veins were full and interrupted. The nose and ears were normal. The tongue was swollen from biting. The pharynx was markedly injected. The neck was slightly rigid. The chest, heart and lungs were normal. The abdomen was distended. The extremities showed spasticity, more marked on the right. The deep reflexes were hyperactive, more so on the right. Abdominal reflexes were not elicited. The Babinski sign was positive bilaterally.

The blood count showed 29,600 leukocytes, of which 75 per cent were polymorphonuclears, 22 per cent lymphocytes and 3 per cent mononuclears.

The spinal fluid was removed under slightly increased pressure; it was slightly cloudy and contained 200 cells per cubic millimeter, 85 per cent of which were lymphocytes. Gram-positive diplococci were seen on direct smear. The globulin was ++, and Fehling's solution was reduced.

The spinal fluid removed four hours later contained 4,900,000 cells per cubic millimeter, 90 per cent being polymorphonuclears.

The patient did not respond to treatment, but died on the day of admission. The clinical impression was that of cavernous sinus thrombosis, left orbital cellulitis, meningitis and acute pharyngitis.

Postmortem examination showed that the left eyeball was normal. On removing the calvarium, the epidural surface was normal. The meningeal vessels were congested. The brain was edematous. The subarachnoid of the anterior and inferior surface of the left temporal lobe contained thick, yellow-green, creamy pus in a tract about 4 cm. wide. This could be traced to the cerebral surface of the frontal lobe, where there was a large collection of pus. On the adjacent right hemisphere, a similar but less abundant exudate was seen. In the center of the inferior surface of the left frontal lobe was a mass of granulation tissue about 1 cm. in diameter, from which thick, yellow-gray pus exuded on pressure. No pus was found at the base of the brain. On the orbital plate of the left frontal bone, immediately beneath the granulation tissue described, was a defect about 5 mm. in diameter, surrounded by a few fragments of bone, the result of perforation. The dura mater covering this area was congested, and on its superior surface there was about 2 cc. of thick, gray-yellow pus. On removal of the dura, pus could be expressed through the orbital opening by pressure on the eyeball. Immediately beneath the perforation, in the orbital fat, was a small abscess containing fluid pus; lodged just beneath the fracture were fragments of pencil and a piece of lead.

#### DISCUSSION

MAX LEDERER: There are some points to be emphasized here. First, only a very close examination of the left eyelid disclosed a scar. Apparently the wound had healed by primary union, and there was certainly no infection of the tissues of the eyelid. Second, the specimen being shown does not represent the actual picture, because in trying to fix the end of the pencil in the soft tissues, the fragments of bone were dislodged and lost. Where there is now a hole in the



orbital plate, that place was occupied by fragments of the bone. Third, from a practical standpoint, Dr. Goodman did not mention that there was a laceration of the inferior surface of the frontal lobe, where the point of the pencil had lacerated the brain tissue as well, which later became the site of the superficial abscess.

This case also emphasizes the necessity in all these instances of making a roentgen examination of the skull or orbit. The patient was first seen in the hospital to which he had immediately been taken; the wound was then sutured and the child discharged, with the sequelae that have been demonstrated.

OSTEOGENIC SARCOMA-LIKE TUMOR OF THE METACARPAL BONE. HENRY L. JAFFE and (by invitation) LEO MAYER.

A tumor of the fourth metacarpal bone was described. It began when the patient, a girl, was 12 years of age. After growing slowly for almost three years, it began to grow rapidly. The tumor mass was removed; it measured 10 by 6.5 by 6 cm. Only the articular head of the metacarpal remained. The tumor was of such consistency that it could be cut with a knife, but it was quite granular and firm. Histologically it showed a marked production of osteoid tissue and also the formation of trabeculae of normal, new-formed bone. The osteoid tissue arose from cells that had many of the features of osteoblasts. The tumor has not recurred one and a half years after operation, though the histologic appearance suggests on casual examination the diagnosis of osteogenic sarcoma. It is believed that this tumor is an osteoblastic, osteoid-forming tumor of slow growth, and that the prognosis is good. It is therefore related to the osteoid chondroma of Virchow.

DISCUSSION

PAUL KLEMPERER: This is a very unusual type of tumor and has many interesting points. I should like to say that I admire the courage of Dr. Jaffe, for calling this tumor from the very first nonmalignant. I think that most of us would have made the diagnosis of a malignant osteogenic sarcoma. If I understood Dr. Jaffe correctly, there was one point that caused him to consider this tumor nonmalignant, from the histologic aspect, and that was the presence of the osteoblasts. I think one is struck by the uniformity of the cell type, which coincides very well with the osteoblasts of the normal bone, and this may be then actually a differential point that might help in future cases to give a better prognosis than one would if one should call this tumor an osteogenic sarcoma. That it is an osteogenic tumor I think Dr. Jaffe does not doubt. It originates in the mesenchyme of the bone, and this cellular structure and intercellular substance are found only in bone.

The question arises whether tumors of the osseous mesenchyme, which have such a definite maturation, like the production of osteoblasts, are less malignant than other tumors. Some years ago I saw a tumor in certain respects similar to this, in which the cell type was such that I could only identify it with the osteoblasts, and here also the uniformity of the cell type was very striking. In this case it was not a tumor of a short bone, but of the humerus. The end-result was similar to that in this case. It occurred in a boy of 15 years, who three and a half years ago was operated on because of the tumor in the head of the humerus. Only the tumor was resected. No radical operation was performed, the tumor being excised with a wide margin. The histologic picture was that of a very cellular tumor with one cell type predominating. This patient is today in perfect health and has no metastases.

Can Dr. Jaffe tell us how long one can expect metastases from an osteogenic sarcoma after removal of the tumor—whether there is sometimes a very long latent period or not? As far as I recall, I think usually the period is short, and that metastases appear very shortly after the onset of the disease. In this case of four years' duration, is it correct to assume that no metastases will occur?

Furthermore, I would like to know whether what we used to call osteoid sarcoma might not be the same type of tumor. I believe that osteoid sarcoma was generally considered not as malignant as osteochondrosarcoma, so this question has to be asked in order to classify this tumor properly. It might be identical with the osteoid sarcoma of old and remarkable mainly on account of its peculiar localization.

FRANCIS CARTER WOOD: I have seen a tumor of the antrum which in some ways resembled what Dr. Jaffe has shown. It was submitted to the members of the Codman Committee, most of whom believed it benign. The growth filled the antrum and was resected as a sarcoma. I made the diagnosis of sarcoma, but the patient is still well.

HENRY L. JAFFE (closing): I am of the same opinion concerning osteogenic sarcoma as Dr. Klemperer—that it generally metastasizes quickly; four and a half years is a long time. I think that we may now expect this girl to make a complete recovery.

I am not certain that I know definitely what the osteoid sarcoma of Virchow is. It is difficult to decide from what is described in the old literature. These tumors are described as containing cartilage, and others describe lesions resembling periostitis. I have had specimens of osteoperiostitis sent in suspected of being Virchow's osteoid tumor, which of course they are not.

I believe that the thing of practical importance in connection with osteoblastic tumors of the hands and feet is the question of amputation. I am not considering bones such as the os calcis. I have been unable to find in the literature cases that have been followed for any period of time. Aside from the two cases described by Bergstrand, Handl described two cases in 1906. These were reported shortly after removal of the tumors, and his report is not accompanied by photographs or drawings, and this makes it difficult to judge independently what he was dealing with. Buffalini, some years later, described a tumor that seems to have arisen from a metacarpal bone. This was possibly of the mixed cell type, but it was also described very shortly after removal.

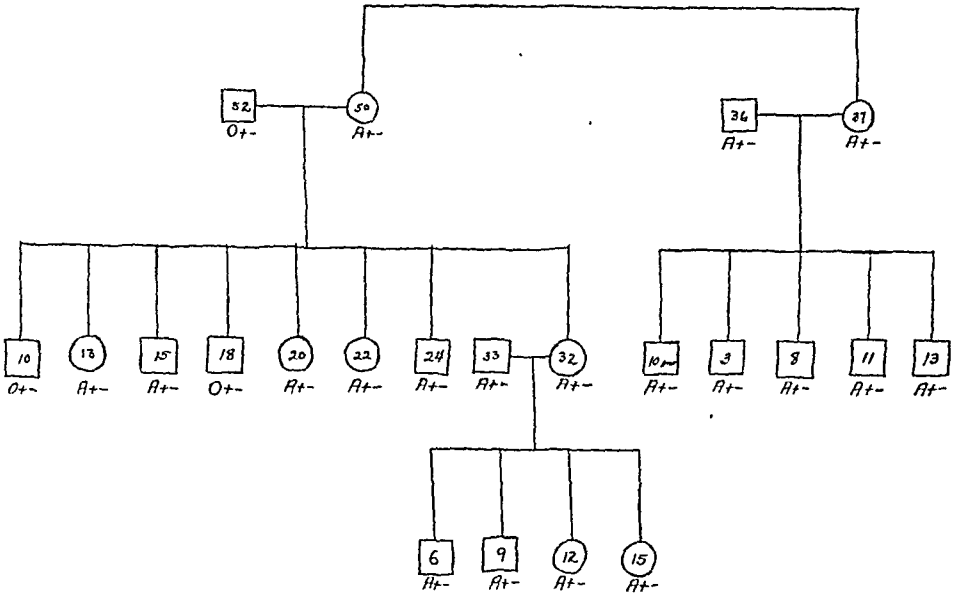
#### HEREDITY OF THE AGGLUTINOGENS M AND N OF LANDSTEINER AND LEVINE, WITH PARTICULAR REFERENCE TO THE DETERMINATION OF PATERNITY. ALEXANDER S. WIENER AND MAX LEDERER.

By means of immune rabbit agglutinins, Landsteiner and Levine have demonstrated the existence in human red blood cells of several agglutinogens, designated by them as M, N and P, that are independent of the agglutinogens A and B, and have succeeded in dividing all human blood into at least thirty-six distinct types. Of the newer agglutinogens, Landsteiner and Levine studied the two agglutinogens M and N in greater detail, and these agglutinogens are the subject of this paper.

Landsteiner and Levine have demonstrated that the agglutinogens M and N are inherited as mendelian dominants and have also proposed a theory according to which the inheritance of these agglutinogens depends on a single pair of allelomorphous genes, M and N, located in a certain pair of chromosomes. Any person could therefore have in that pair of chromosomes any one of the following combinations of genes: MM, MN or NN. And, corresponding to the genotypes, MM, MN and NN, we have three phenotypes, termed by Landsteiner and Levine  $M+N-$ ,  $M+N+$  and  $M-N+$ , respectively. The theory, therefore, accounts for the nonexistence of the type  $M-N-$ . On the basis of this theory, the expected values for the types of offspring are: from cross  $M+N+$  by  $M+N+$ ,  $M+N+$  50 per cent,  $M+N-$  25 per cent and  $M-N+$  25 per cent; from cross  $M+N+$  by  $M-N+$ ,  $M+N+$  50 per cent and  $M-N+$  50 per cent; from cross  $M+N+$  by  $M+N-$ ,  $M+N+$  50 per cent and  $M+N-$  50 per cent; from cross  $M+N-$  by  $M-N+$ ,  $M+N+$  100 per cent; from cross  $M+N-$  by  $M+N-$ ,  $M+N-$  100 per cent, and from cross  $M-N+$  by  $M-N+$ ,  $M-N+$  100 per cent.

The results of a study of 131 families with 642 children are shown in table 1. Two apparent exceptions to the theory of Landsteiner and Levine were found. In one family, the father and mother were both of type  $M + N -$ , but one of the children was of type  $M + N +$ . In another family, the father belonged to type  $M - N +$ , the mother to type  $M + N +$ , and one of the children to type  $M + N -$ . In both cases there is a possibility of illegitimacy, however, especially in considering the fact that as many as 642 children were examined in this study.

The same families that were examined for M and N were also examined for the heredity of the agglutinogens A and B. There was one apparent exception



Ross-Jagofsky-Shapiro family.

TABLE 1.—Heredity of Agglutinogens M and N of Landsteiner and Levine

Types of Parents	Number of Families	Children of Types			Totals
		M+ N+	M+ N-	M- N+	
M+ N+ × M+ N+	25	58	29	29	116
M+ N+ × M- N+	36	83	1*	92	176
M+ N+ × M+ N-	43	119	97	0	216
M+ N- × M- N+	10	46	0	0	46
M+ N- × M+ N-	14	1*	63	0	69
M- N+ × M- N+	3	0	0	19	19
Totals.....	131	307	195	140	642

\* A question of illegitimacy arises here.

to the Bernstein theory of heredity of the Landsteiner blood groups, namely, a family in which the father belonged to group AB, the mother to group B and one of the children to group O. Since the exception here is on the paternal side, the possibility of illegitimacy cannot be excluded. Our study, therefore, produced additional evidence in favor of the Bernstein theory.

One of the families examined was studied into the third generation. The result of this study is shown in the accompanying diagram. The family consisted of 22 persons living in one large three-family house. The remarkable observation was that of these 22 persons living in one house, 19 were of type A + — and three of type O + —. Thus, of 22 persons, 19 belonged to one type and 3 to

another. Furthermore, von Dungern and Hirzfeld in 1910 demonstrated the existence of two different subgroups of group A cells, which have been designated as  $A_1$  and  $A_2$  by Landsteiner and Levine, of which  $A_1$  is the more frequent subgroup. The 19 group A persons in the Ross-Jagofsky-Shapiro family belonged to subgroup  $A_1$ .

The results of this study, with the results published by Landsteiner and Levine, and by Schiff, indicate, as has already been pointed out by Landsteiner and Levine, that the agglutinogens M and N can be used medicolegally for the determination of nonpaternity, as is shown in table 2.

It is of interest to know how frequently nonpaternity can be proved when the putative father is not the true father. It has been calculated that in one sixth of such cases, nonpaternity can be proved by means of agglutinogens M and N alone. This is equal to the chances of proving nonpaternity by means of agglutinogens A and B, so that the discovery of Landsteiner and Levine has doubled the number of cases of questioned paternity that can be solved.

TABLE 2.—*Heredity of the Agglutinogens of M and N of Landsteiner and Levine*

Types of Parents	Types of Children Possible	Types of Children Not Possible
M+ N+ × M+ N+	M+ N+, M+ N-, M- N+	
M+ N+ × M- N+	M+ N+, M- N+	M+ N-
M+ N+ × M+ N-	M+ N+, M+ N-	M- N+
M+ N- × M- N+	M+ N+	M+ N-, M- N+
M+ N- × M+ N-	M+ N-	M+ N+, M- N+
M- N+ × M- N+	M- N+	M+ N+, M+ N-

The new-found agglutinogens can also be used in cases in which new-born infants have been accidentally interchanged, as happened in a recent case in Chicago. Fully two thirds of such cases can be solved by the combined use of the agglutinogens A, B, M and N.

#### DISCUSSION

MAX LEDERER: I should like to emphasize, as I did about a year ago, the medicolegal importance of this work, which apparently has not been recognized in this country. In Germany, Norway, Switzerland and Denmark this method of testing has been accepted in the courts in cases in which there exists illegitimacy, and the courts have made it mandatory that these tests shall be done before the trial takes place, the reasons being twofold: first, it saves the government the expense of conducting a trial, and second, it prevents perjury. The latest statistics on the subject that I have been able to collect show that now there have been tests in 5,512 cases in the German courts with the old grouping method, but not with the M and N method, and in about 448 cases it was shown definitely that the accused person could not be the father of the child. That is quite a large number of cases, and certainly testifies to the importance of the method.

In a recent case, the question was put to Dr. Wiener, whether the husband or a second man was the father of a child. As both men belonged to group A, the Landsteiner blood groups did not help solve the problem. By examination of the bloods for M and N factors of the mother, the child and the two men, the second man could be excluded as father of the child, and the legitimacy of the child was thus established.

There was also an application of this test in cases of murder. I need not go over the details here. It is indeed surprising that this country, progressive as it is in medical work, should so far have done nothing to apply these tests medicolegally. The A and B method alone has been used in a few instances, as in the Chicago case. However, there are any number of cases that occur in large cities, in which it is necessary for the corporation counsel to obtain all the information possible in order to decide whether a given man is the father of a

child or not, and if one takes into consideration the saving of the expense of the numerous trials that take place for this purpose, it certainly seems worth while to incorporate this test into our medicolegal procedures.

SILIK H. POLAYES: From the point of view of transfusions, although there are no normal agglutinins for the agglutinogens M and N, it is possible that the same thing that happens in rabbit's blood may happen in human blood, if the recipient who does not possess these agglutinogens receives blood from a person whose blood contains either of them. In other words, the first transfusion may stimulate the production of agglutinins specific to agglutinogens M and N, so that on subsequent transfusions it seems possible for a reaction to occur between the newly developed agglutinins and the foreign agglutinogens M and N.

ALEXANDER S. WIENER (closing the discussion): I might add that there are two subgroups of A, namely, A<sub>1</sub> and A<sub>2</sub>, and in addition the agglutinin P which I mentioned. The heredity of these agglutinogens has also been studied; they are definitely inherited, but because of the technical difficulty in testing for these substances, the heredity is not perfectly worked out yet, and the time is not ripe for their medicolegal application. The possibility remains that by the immunization of animals besides rabbits with human blood, more and more agglutinogens may be discovered as time goes on, so that the percentage of cases of nonpaternity proved without the aid of any evidence but the examination of the bloods, instead of being 33, may be increased to 50 or 75, or perhaps at some time to the ideal of 100 per cent. Furthermore, with just the agglutinogens that I have mentioned, namely, A<sub>1</sub>, A<sub>2</sub>, B, M, N and P, we can distinguish thirty-six different types of human blood. Therefore, in a medicolegal case, in which a question of the identification of a blood stain arises, when the blood stain might belong to one of two persons, the chances of the two persons belonging to the same one of the thirty-six types are very small, so that the chances of identifying blood stains in such cases are good.

#### LESIONS OF THE BRAIN IN INFLUENZA. IRVING J. SANDS (by invitation).

Nervous and mental complications are quite common in influenza. They are essentially due to changes occurring in the nervous system. The brain changes in the 1889-1891 epidemic have been described in the literature as "hemorrhagic encephalitis." The epidemic of 1917-1919 was so intimately associated with epidemic encephalitis that all the lesions of the brain occurring in that epidemic of influenza have been described under the heading of epidemic encephalitis. Four cases occurring in the recent epidemic of influenza have been examined post mortem at the Jewish Hospital of Brooklyn and studied anatomically. There is no doubt of their being cases of influenza.

Grossly, the brains appeared reddened and congested, and the veins were distended with blood. There were numerous small punctate pial hemorrhages present. The dura apparently was normal. The pia was congested and showed infiltration with lymphocytes, plasma cells and a few polymorphonuclear cells. The pial vessels were engorged with blood. The nerve cells showed marked cloudy swelling, chromatolysis, satellitosis and neuronophagia. There were numerous areas where there was a great loss of nerve cells, and the latter were replaced with glia cells. The blood vessels showed inflammatory changes, the Virchow-Robin spaces being filled with lymphocytes and plasma cells. There were numerous areas where there was considerable blood around these vessels. The changes were most marked in the region of the third ventricle and in the corpus striatum. In one case there was an extensive subarachnoid hemorrhage, caused apparently by a massive cerebral hemorrhage. The changes were also found in the white matter. In one case, the chief seat of pathologic alteration was in the medulla.

Case 1 was that of a girl of 14 months, who became ill with a cough and running nose, and two days later was admitted to the hospital. She was cyanotic and dyspneic and appeared toxic. She showed signs of congestion in the base of the left lung and leukopenia. Death occurred three hours after admission.

Postmortem examination showed the characteristic red and congested condition of the pharynx, larynx, trachea and bronchi. The lungs showed hemorrhagic areas, and there was considerable hemorrhage in the alveoli. A purulent process was found in the bronchi. There were numerous abscesses in the lungs. There were petechial hemorrhages in the kidneys. The brain showed the changes described.

Case 2 was that of a man complaining of abdominal pain on admission to the hospital. He showed general arteriosclerosis and an icteric tinge to the skin. He lapsed into coma and died a day after admission. Postmortem examination showed the reddened condition of the respiratory tract and the hemorrhagic condition in the lungs. Coronary sclerosis, chronic cholelithiasis and myofibrosis cordis were found. The brain showed the changes described, and there was a moderate subarachnoid hemorrhage over the parietal lobe.

In case 3, a high temperature and respiratory difficulty suddenly developed in a girl of 4 months. On admission to the hospital, she had two general convulsions. There was dullness at the bases of both lungs. Death occurred two days after the onset of the illness. Postmortem examination again showed the typical changes in the larynx, pharynx, trachea and bronchi. The lungs showed edema and congestion, and hemorrhagic exudate in the alveoli. The brain showed the changes described. There was a large subdural clot in the left middle fossa.

Case 4 was that of a 48 year old woman, who had nursed four members of her family in influenza. She suddenly complained of weakness and headache. The following day she went to bed and became drowsy. The next day she showed marked rigidity of the neck, depressed deep reflexes, slow pulse and a temperature of 101 F. The diagnosis of subarachnoid hemorrhage was made, and on admission to the hospital a bloody spinal fluid confirmed the diagnosis. She lapsed into deep coma and died two hours after admission. Postmortem examination disclosed a congested, hemorrhagic condition of the trachea, bronchi and lungs. There was marked subarachnoid hemorrhage, covering the entire right hemisphere, more marked at the anterior two-thirds, and there was a similar, though lesser, hemorrhage on the left side. The great cisterns were filled with blood. There was a massive cerebral hemorrhage, measuring 3 by 4 by 5 cm., in the left frontal lobe, which communicated with the subarachnoid space, but which did not communicate with the ventricles. The rest of the brain showed the changes described.

The cause of influenza is unknown. There are many who believe that the Pfeiffer bacillus is responsible. There are others who maintain that it has nothing to do with influenza. The cause of epidemic encephalitis is unknown. Many maintain that it is caused by a filtrable virus; some claim that it is due to a streptococcus, and many believe that it is caused by a toxin the organisms producing which are situated in some other part of the body. Many are convinced that there is a close relationship between influenza and epidemic encephalitis. There is a group of observers who believe that it is caused by the same noxious agent. The relationship between epidemic encephalitis and influenza is still a subject of great controversy and speculation.

#### DISCUSSION

MAX LEDERER: One of the most difficult things in evaluating the information obtained is the primary diagnosis. Are we dealing with influenza or not? This difficulty of diagnosis was very well emphasized during the recent epidemic of influenza. There are those of us who have had the fortunate experience (from our standpoint) of performing autopsies in quite a number of cases of influenza during the epidemic of 1918-1919, and we were thoroughly impressed with the pictures those cases presented, and those who have not seen material of that type are skeptical as to whether we are dealing with the same condition now. In the beginning of this last epidemic I began to watch for these cases; we performed autopsies in about fourteen, in all of which was shown the one outstanding sign on which I place a great deal of importance, namely, the intense reddening of

the trachea and the bronchi, and perhaps the larynx as well, with ulceration and hemorrhage, occasionally accompanied by a dark green, shaggy membrane. Of course, the other respiratory lesions that occurred were not so constant, and I suspect that they were not so constant because of the superimposed processes that occurred, such as empyematous abscesses due to staphylococci particularly, and in some instances in which the lesion in the upper part of the lung was relatively mild the patients died of something else, or as in this one case, of a hemorrhage in the brain. In this case Dr. Sands told the story as it occurred as far as the patient was concerned from the standpoint of the history of the disease, but not from that of the diagnosis. The latter was made at the autopsy table, and Dr. Sands went back and obtained the story later on, that the patient had been nursing patients with influenza. We originally suspected that she had been suffering from a fracture of the skull, which made it a medical examiner's case.

After we had seen these fourteen or fifteen cases, I had my colleagues observe the pulmonary cases from a different point of view, and we were able to point out regularly, after the epidemic was over, that there was no reddening of the trachea, or very little, and I was able to convince most of my colleagues that the earlier cases belonged to the influenza group. They are being studied now for further report.

The lesions of the brain are of great interest, and Dr. Sands brings up the important point of the relationship of encephalitis to influenza, which is very suggestive, since after the 1918 epidemic of influenza the epidemic of encephalitis followed so promptly.

MAX TRUBEK: Was the possibility of traumatic cerebral hemorrhage thought of to explain the lesions in the last case? The multiple punctate cerebral hemorrhages appear not unlike those sometimes occurring after cranial injury, often without fracture of the skull. This probability came to mind even before the discussion showed that the fall with possible fracture of the skull was considered as a cause of death, before the autopsy.

IRVING SANDS (closing the discussion): The picture is entirely different. We find multiple punctate hemorrhages in traumatic encephalopathies, but we do not find any inflammatory areas around the capillaries. We do not see lymphocytic or plasma cell infiltration. That is the outstanding difference. Moreover, there are not found the typical toxic changes in the nerve cells.

## Book Reviews

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THE RENAL LESION IN BRIGHT'S DISEASE. By THOMAS ADDIS, Professor of Medicine, Stanford University Medical School, San Francisco, and JEAN OLIVER, Professor of Pathology, The Long Island College of Medicine, Brooklyn; Formerly Professor of Pathology, Stanford University, San Francisco. Cloth. Price, \$16 net. Pp. 650, with 160 full page illustrations, 21 text illustrations and a large folding table. New York: Paul B. Hoeber, Inc., 1931.

This elaborate monograph presents in detail the results of a thorough, well considered study, extending over many years under the same general conditions, of the correlation between certain clinical phenomena and the anatomic changes in the kidneys in Bright's disease. As indicated by the title, only the renal lesions of Bright's disease are considered. By Bright's disease the authors mean bilateral, nonsuppurative renal disease associated with proteinuria. Their immediate problem was to determine the relations between the changes in the urine and the structural changes in the kidneys. Following the introductory chapter are three chapters dealing with the clinical methods used in the investigation, clinical definitions and a clinical classification. Probably never before has the urine of a series of cases of Bright's disease been studied so fully and in such detail with standardized quantitative methods as in this investigation. The next two chapters describe the methods and definitions employed in the study of the kidneys. Chapter 7, by far the longest, discusses the results of the clinical and anatomic observations of seventy-two cases of Bright's disease by the methods described. Two large photomicrographs,  $\times 25$  and  $\times 125$ , illustrate the renal lesion in each case. A folding chart in a pocket on the inside of the first cover page gives a summary of the results in each case. Then follow chapters on comparison and correlation of the results of the clinical and structural observations, on the theoretical description of the course and sequence of the morbid processes in Bright's disease and on a classification and theory of Bright's disease. On the basis of correspondence between the urinary changes and the structural changes in the kidneys, the following classification of Bright's disease is set up: hemorrhagic, degenerative and arteriosclerotic. These terms are regarded now as self-explanatory. Generally speaking, these forms of Bright's disease can be recognized clinically by the results of proper examination of the urine. One is impressed with the complete grasp of the problems involved and with the thoroughness and objectiveness of the observations and the soundness of the interpretations.

The illustrations are excellent. The book is printed on heavy paper with wide spacing. It weighs more than 2,700 Gm. (6 pounds +), and the question as to whether this is the best form of book for the purpose comes up. "Fitness to its subject is the first quality of a wellmade book." However this question may be answered, the great scientific value of the contents recorded will not be affected. What next of Bright's disease? Experiment. "The correlation of function and anatomical structure which has not been touched on in this discussion at all, and which is the basic and all important part of the problem, is still a complete mystery. It is by experimentation, under controlled and simplified conditions, that this ultimate phase must be attacked. And it is from such endeavor . . . that a more satisfactory theory and classification of Bright's disease will eventually evolve."

TEXT-BOOK OF PATHOLOGY. By ROBERT MUIR, M.A., M.D., Sc.D., LL.D., F.R.S., Professor of Pathology, University of Glasgow, Pathologist of the Western Infirmary, Glasgow. Second edition. Price, \$14. Pp. 872, with 501 illustrations. New York: Longmans, Green & Company, 1931.

This is the second revised edition of a textbook that first appeared in 1924. The value of the work is attested by the six printings through which the first edition has since gone. In the one volume, general and systemic pathology are discussed, but, as the author states in the preface to the first edition, these terms



are not used as it seemed inadvisable in a book of this nature to draw any sharp distinctions. The chapter on inflammation is especially clear and lucid, and it is well illustrated with many excellent reproductions. It is interesting that in classifying tumors, the groups histioma and cytoma, rather unusual terms in the American literature, are frequently used. In the chapter dealing with the brain, one finds its tumors classified as fibro-endotheliomas or meningiomas, neurofibromas and gliomas. Although the terms ganglioma and neuro-epithelioma are mentioned, no subdivision of the tumors of the glioma group is given. However, in an earlier general chapter on tumor, there is a discussion of the various tumors of the glioma group. The presentation of electrocardiographic tracings in the chapter on the heart conveys to the student the conception of applied cardiac pathology. This discussion is somewhat more detailed and broader in scope than that usually found in textbooks of pathology. Fibrosis of the myocardium and inflammatory changes are strictly differentiated. It is interesting to note that syphilis, with the exception of gummatous lesions, is classified among the fibroses and not as an inflammation. In many instances throughout the book reference is made to the disturbances of function as well as to the morphologic changes in the various pathologic states.

There are 501 illustrations. The histologic pictures, in general, are excellent; but some of the gross pictures are not as clear and instructive as might be desired. Only very few references are given that refer to larger contributions on the respective subjects. In summarizing, the book is not only a valuable aid for the medical student but also for the pathologist, who will find in it a great deal of information concerning modern nomenclature and conceptions of disease.

**A TEXT-BOOK OF PATHOLOGY.** By FRANCIS DELAFIELD, M.D., LL.D., Sometime Professor of the Practice of Medicine, College of Physicians and Surgeons, Columbia University, New York, and T. MITCHELL PRUDDEN, M.D., LL.D., Sometime Professor of Pathology, College of Physicians and Surgeons, Columbia University, New York. Fifteenth edition. Revised by FRANCIS CARTER WOOD, M.D., Director of the Pathological Department, St. Luke's Hospital, New York; Director of the Institute of Cancer Research, Columbia University, New York. Price, \$10 net. Pp. 1,339, with 20 full-page plates and 830 illustrations in black and in colors. New York: William Wood & Company, 1931.

A brief history of this book is given in connection with the notice of the thirteenth edition (*ARCH. PATH.* 1:496, 1926). The first edition was published in 1872 as a "Hand-Book of Postmortem Examination and Morbid Anatomy," by Francis Delafield. Hence the book is about to complete the unique record of fifty years of usefulness. At least since 1901, its avowed purpose has been to meet the need of medical students and physicians for a comprehensive, if of necessity somewhat epitomized, presentation of both general and special pathology. A main factor in the success of the book has been its lucid style and an unfailingly firm grasp of principles as well as of details. Herein T. Mitchell Prudden's influence still persists. The successive editions have reflected faithfully the progress in the meantime, and American contributions have received welcome attention. Of the advances in the four years since the previous edition, Dr. Wood, who has carefully revised the last five editions, dwells particularly on the new knowledge concerning various hormones and vitamins. The revision appears to be adequate, and new references, chiefly to monographs, have been added.

**PHYSIOPATHOLOGIE DE LA THYROÏDE. DIAGNOSTIC ET TRAITEMENT DES GOÎTRES.** By LUCIEN DAUTREBANDE de la Fondation Reine-Elisabeth; Membre correspondant de l'Académie Royale de Médecine de Belgique. (Avec la collaboration du Dr. A. LEMORT.) Price, 40 francs. Pp. 326, with 36 illustrations and 40 tables. Paris: Masson & Cie, 1931.

The material in this book is handled in eleven chapters as follows: 1, physiopathology of the thyroid gland; 2, hypothyroidism; 3, endemic goiter; 4, simple goiter; 5 nontoxic adenoma; 6, toxic adenoma; 7, hyperthyroidism without the

usual symptoms; 8, hyperthyroid rheumatism; 9, degenerative changes in adenomas of the thyroid; 10, exophthalmic goiter; 11, treatment for hyperthyroidism.

The book is a summary of the various therapeutic measures employed in the treatment for thyroid disorders, critically reviewed by the open-minded Belgian author.

Pathologic physiology and biochemistry are discussed at length in a lucid and interesting style. Owing to the fact that similar or even identical gross pathologic and histologic changes are seen in the different clinical varieties of diseases of the thyroid, the classification is based mainly on the analysis of clinical findings and on the determination of the basal metabolism; the latter is considered the only absolutely reliable means of diagnosing thyroid disease. The author emphasizes the conception that various types of hyperthyroidism are not so strictly separated as is commonly believed and that there are frequent transitions and borderline cases. Many of the tables and all of the fifty-nine detailed case reports illustrate the effects of the different therapeutic methods. The author champions his method of using small but frequent doses of iodine (for instance: 2 drops, from ten to fifteen times daily). Many so-called iodine-resistant cases responded to this form of medication.

Though the book is primarily intended for the clinician, the pathologist will read with interest the theoretical chapters and will find the thirty-five pages of the bibliography particularly useful.

## Books Received

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APPROVED LABORATORY TECHNIC: CLINICAL, PATHOLOGICAL, BACTERIOLOGICAL, SEROLOGICAL, BIOCHEMICAL, HISTOLOGICAL. Prepared under the Auspices of The American Society of Clinical Pathologists by John A. Kolmer, M.D., D.P.H., D.Sc., LL.D., Professor of Pathology and Bacteriology, Graduate School of Medicine, University of Pennsylvania; Professor of Immunology and Chemotherapy, School of Medicine, Temple University; Head of the Department of Pathology and Bacteriology, Research Institute of Cutaneous Medicine, and Fred Boerner, V.M.D., Associate Professor of Bacteriology, Graduate School of Medicine, University of Pennsylvania, assisted by C. Zent Garber, A.B., M.D., and Committees of the American Society of Clinical Pathologists. Price, \$7.50. With 11 colored plates and 300 illustrations in the text. New York: D. Appleton and Company, 1931.

LEHRBUCH DER SPEZIELLEN PATHOLOGISCHEN ANATOMIE FÜR STUDIERENDE UND AERZTE. Von Dr. Eduard Kaufmann, o. Professor der allgemeinen Pathologie und pathologischen Anatomie an der Universität Göttingen, Geheimer Medizinalrat. Neunte und zehnte, völlig neubearbeitete und stark vermehrte Auflage. Zwei Bände. Erster Band. Price, 55 marks. Pp. 990. Mit 506 Abbildungen im Text und auf färbigen Tafeln, zuallermeist nach Originalzeichnungen des Verfassers. Berlin: W. de Gruyter & Co., 1931.

DIAGNOSIS IN JOINT DISEASE: A CLINICAL AND PATHOLOGICAL STUDY OF ARTHRITIS. By Nathaniel Allison, M.D., F.A.C.S., Professor of Surgery, in Charge of Division of Orthopedic Surgery, University of Chicago, and Ralph K. Ghormley, M.D., Associate in Orthopedic Surgery, Mayo Clinic. From the Orthopedic Service of the Massachusetts General Hospital and the Harvard Medical School (1924-1930). Assisted by the DeLemar Mobile Research Fund. Cloth. Price, \$9. Pp. 196, with 71 illustrations. New York: William Wood & Company, 1931.

FURTHER INVESTIGATIONS ON THE VARIOLA-VACCINIA FLOCCULATION REACTION. By James Craigie and W. J. Tulloch, Medical Research Council, Special Report Series, No. 156. Pp. 129. London: His Majesty's Stationery Office, 1931.

MEDICAL DEPARTMENT, UNITED FRUIT COMPANY. NINETEENTH ANNUAL REPORT. Pp. 276. 1930.

NUTRITIONAL ANAEMIA IN INFANCY WITH SPECIAL REFERENCE TO IRON DEFICIENCY. By Helen M. M. Mackay assisted by Lorel Goodfellow. With a Statistical Appendix by A. Bradford Hill. Medical Research Council, Special Report Series, No. 157. Pp. 125. Price, 2 shillings 5 pence, net. London: His Majesty's Stationery Office, 1931.

A TEXT-BOOK OF PATHOLOGY. By Francis Delafield, M.D., LL.D., and T. Mitchell Prudden, M.D., LL.D. Fifteenth edition revised by Francis Carter Wood, M.D., Director of the Pathological Department, St. Luke's Hospital, New York. Fabrikoid. Price, \$10. Pp. 1339, with 850 illustrations. New York: William Wood & Company, 1931.

ASTHMA AND HAY FEVER IN THEORY AND PRACTICE: Part 1. Hypersensitiveness, Anaphylaxis, Allergy. By Arthur F. Coca, M.D., Professor of Immunology, Cornell University Medical College; Part 2. Asthma. By Matthew Walzer, M.D., Instructor in Applied Immunology, Cornell University Medical College; Part 3. Hay Fever. By August A. Thommen, M.D., Lecturer in Medicine, University and Bellevue Hospital Medical College. Cloth. Price, \$8.50. Pp. 851, with 102 illustrations. Springfield, Ill.: Charles C. Thomas, 1931.

## THE INFECTION OF RABBITS WITH THE TUBERCLE BACILLUS BY WAY OF THE TRACHEA

STUDIES ON THE DEFENSIVE AND METABOLIC APPARATUS  
OF THE LUNGS \*

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BOSTON

The aerogenous infection of animals with the tubercle bacillus is of interest for two reasons: (1) Because of the scantiness of experimental studies of this particular mode of infection with Koch's bacillus. This dearth is the most surprising since the inhalation of the tubercle bacillus is regarded by most observers as being the essential way of infection in man. (2). In connection with the recent views of the pulmonary structure. From previous studies<sup>1</sup> it was inferred that the inflammatory reaction in pathologic conditions of the lungs originates in situ from the cells lying without (i. e., lining) and within the alveolar septums, and also from the peribronchial and the perivascular cells. It then appeared that the cells commonly designated as "alveolar epithelium" are to all appearances not epithelial, but of mesenchymal origin.

This conception, while accepted by one group of observers, was contradicted by another. The studies with the tubercle bacillus were then undertaken in order to find new evidences pertaining to this theory. For, as Maeterlinck<sup>2</sup> expressed it: "To the objection based on an experiment, the best reply of all must be a counter-experiment."

### MATERIALS AND METHODS

The experiments were conducted on full-grown rabbits. A 6 weeks old culture of bovine tubercle bacilli grown on glycerine agar, secured from the American Type Culture Collection, was used. In each case the trachea of the animal was

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\* Read by invitation at the Twenty-Seventh Annual Meeting of the National Tuberculosis Association, Syracuse, N. Y., May 11-14, 1931.

\* This work was made possible through the assistance of Prof. Harvey Cushing.

1. Fried, B. M.: The Origin of Histiocytes (Macrophages) in the Lungs, *Arch. Path.* **3**:751, 1927; The Defensive and Metabolic Apparatus of the Lung; The Lungs and the Macrophage System, *ibid.* **6**:1008, 1928; The Infection of Rabbits with the Anthrax Bacillus by Way of the Trachea, *ibid.* **10**:213, 1930.

2. Maeterlinck, Maurice: *La vie des abeilles*, Paris, Fasquelle.

exposed in a manner described previously,<sup>3</sup> and 0.5 mg. of the acid-fast bacilli emulsified in 1 cc. of a physiologic solution of sodium chloride was injected into the lung via the trachea.

The animals were killed by an air embolus at intervals of from one minute to several weeks after the intratracheal injections.

Tissues were fixed in a 10 per cent solution of formaldehyde, in Zenker's fluid and in alcohol. Paraffin and frozen sections were stained with hematoxylin and eosin, with methylene blue and eosin and with Ziehl-Neelsen's stain for acid-fast bacilli.

#### OBSERVATIONS

*After One Minute.*—When the lungs were removed from the thoracic cavity about one minute after the intratracheal injection of the tubercle bacilli, they

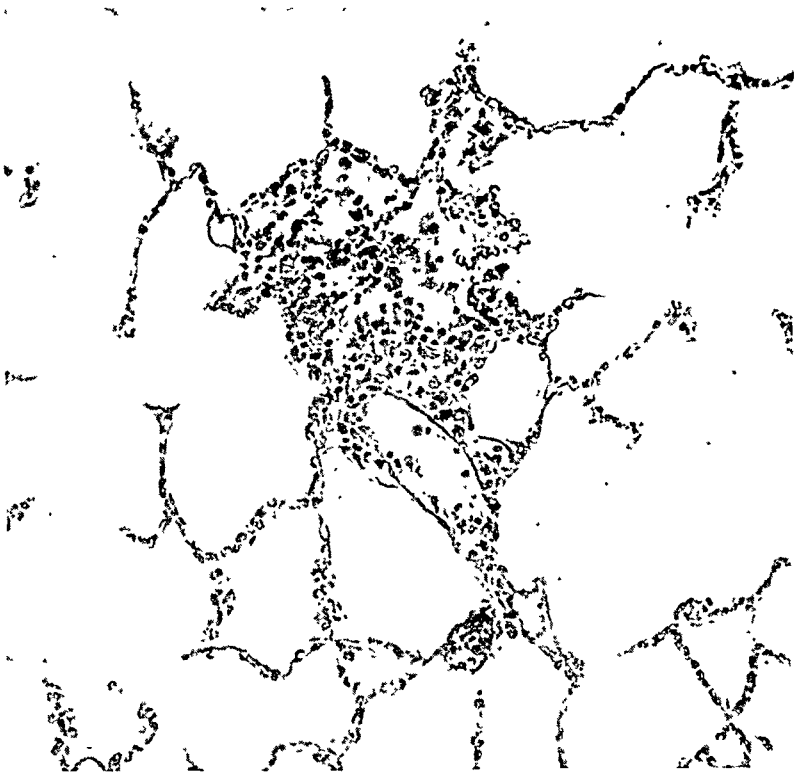


Fig. 1.—The reaction found in the pulmonary alveolus of the rabbit one minute after intratracheal injection of bovine tubercle bacilli. Methylene blue and eosin;  $\times 175$ .

showed no gross changes. However, sections studied with the microscope revealed an early remarkable response of the pulmonary tissue as follows:

Just as in the experiments with vital staining, in which the dye impregnated the lung in patches only, so the tubercle bacilli produced multiple foci of lesions, which under the lenses of low power were conspicuous as groups of cells lying close to the septums (figs. 1, 2 and 3). The alveoli, which vary in shape and size, could be easily made out, showing moderately distended septal capillaries containing erythrocytes arranged in one single file. With the high-power lenses the following details were remarkable (figs. 2 and 3): Most of the cells that had so rapidly

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3. Fried, B. M., and Proctor, E. E.: The Appearance of Specific Antibodies in the Serum of Rabbits by Intratracheal and Intravenous Injections of Living Tubercle Bacillus, *Proc. Soc. Exper. Biol. & Med.* **21**:396, 1924.

proliferated remained in close connection with the wall of the air sac. Indeed, they did not represent a continuous membrane, but were discontinuous, forming agglomerations here and there. A few cells were seen to lie free in the alveolar lumen, and once in a great while the liberated cells were in mitosis, whereas amitosis could be seen in the sessile cells only.

The cells varied in size from that of a medium-sized lymphocyte to that of a voluminous phagocyte. In the smaller cells the ill defined cytoplasm was barely visible, and the nuclei were irregularly round and contained coarse chromatin

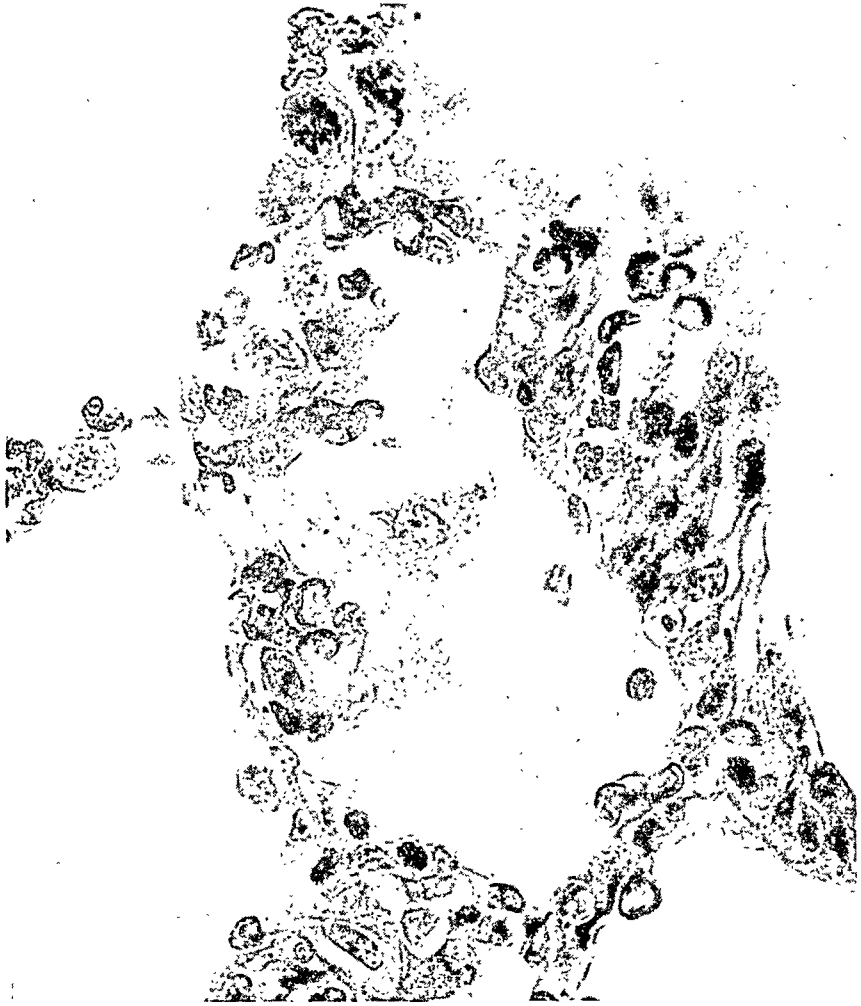


Fig. 2.—The morphology of the proliferated cells lining the air sac of the rabbit one minute after aerogenous infection with Koch's bacillus. Mitotic figures are seen in the upper segment of the photomicrograph. Methylene blue and eosin;  $\times 850$ .

particles. There were numerous transitions between the small and the large varieties of cells, this being shown by variations in the size of the cytoplasm, but not by the nucleus, which remained practically unchanged. In the large cells the cytoplasm was irregular and foamy, being two or three times larger than the nucleus, which was pushed to the cellular periphery.

The cells that "stuck" to the wall of the air vesicle occasionally contained an acid-fast bacillus in their cytoplasm, while those lying free were often loaded with Koch's bacilli.

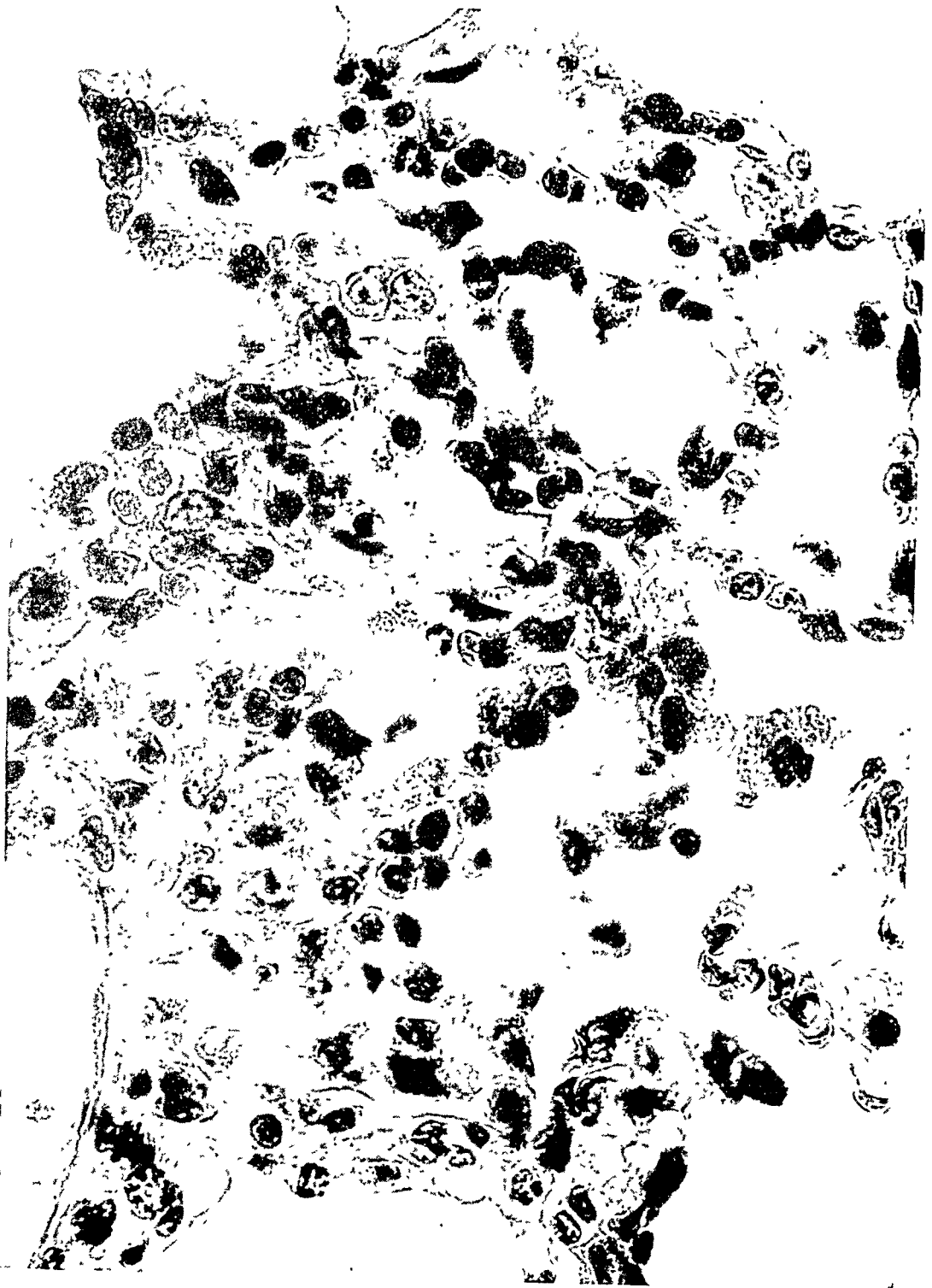


Fig. 3.—A section from the lung of a rabbit that died one minute after intratracheal injection of acid-fast bacilli. The morphology of the fixed and of the wandering cells should be noticed. Methylene blue and eosin;  $\times 380$ .

Granulocytes could be seen rarely between the groups of large mononuclear cells. An occasional one contained a tubercle bacillus, and sometimes a polymorphonuclear white cell could be found to be, in its turn, phagocytosed by a large mononuclear cell.

The alveolar lumen contained no serum, fibrin or red cells. The endothelium of the septal capillaries showed no changes and no phagocytosis. Likewise, no changes were found in the large blood vessels.

However, the reaction to the tubercle bacilli appeared microscopically to be focal; a search revealed that "irregularities" were also conspicuous in the vicinity

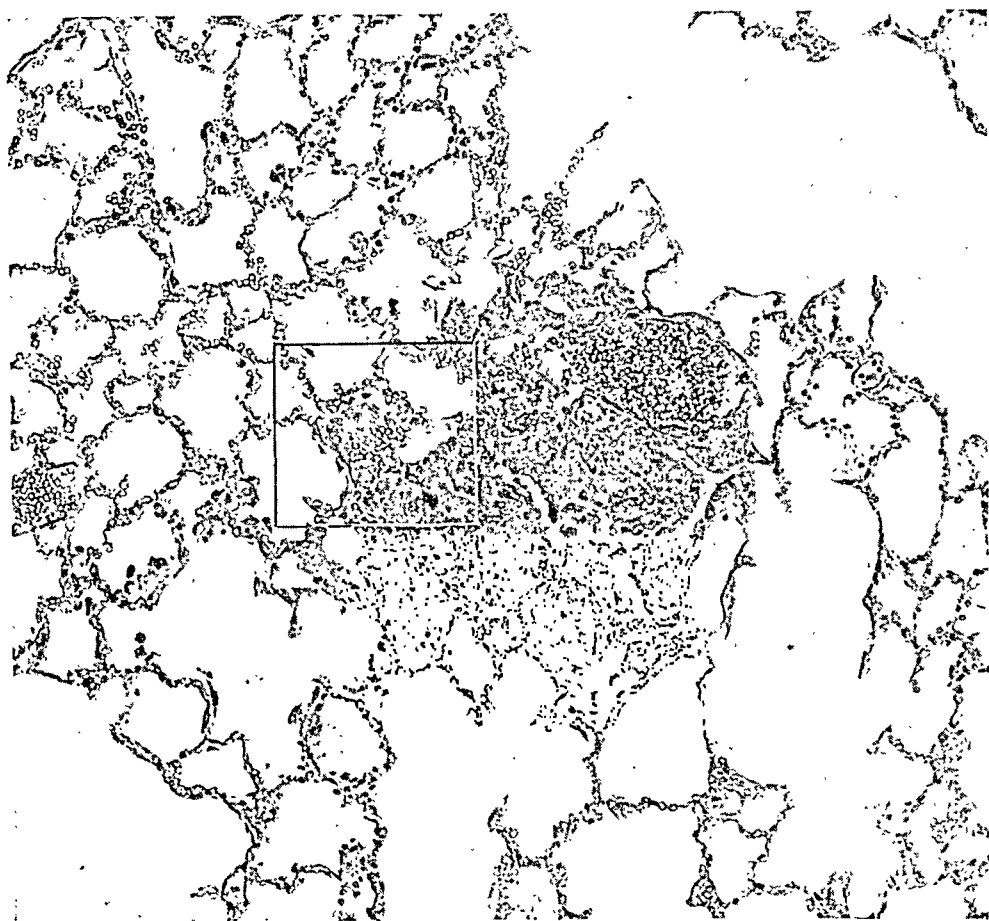


Fig. 4.—The aspect of a "miliary granule" formed in the rabbit's lung five minutes after injection of tubercle bacilli via the trachea. The proliferated alveolar "epithelial" cells are transformed into epithelioid cells (parenchymatous alveolitis). The area within the quadrant is shown under high power magnification in figure 5. Methylene blue and eosin;  $\times 150$ .

of the just described minute "granulomas." Thus, here, too, there occurred an increase in the number of the cells alongside the wall of the air sac, and cells lying free in the respiratory vesicle could also be seen scattered here and there.

Whereas the aspect of the smaller cells was that of the lymphomonocytoid variety (and these cells are apparently the small nucleated cells of the respiratory alveolus), that of the larger ones, which are in all likelihood their direct descendants, was that of fixed or of wandering macrophages.

The lymphoid tissue showed nothing worthy of note.



A thorough search failed to reveal the existence of a so-called "membrana epithelia," for the cells that displayed the described activities "lined" the septums and therefore directly faced the lumen of the air sac.

*After Five Minutes.*—When the rabbit's lung was examined five minutes after the intratracheal injection, its gross appearance was likewise not remarkable. The

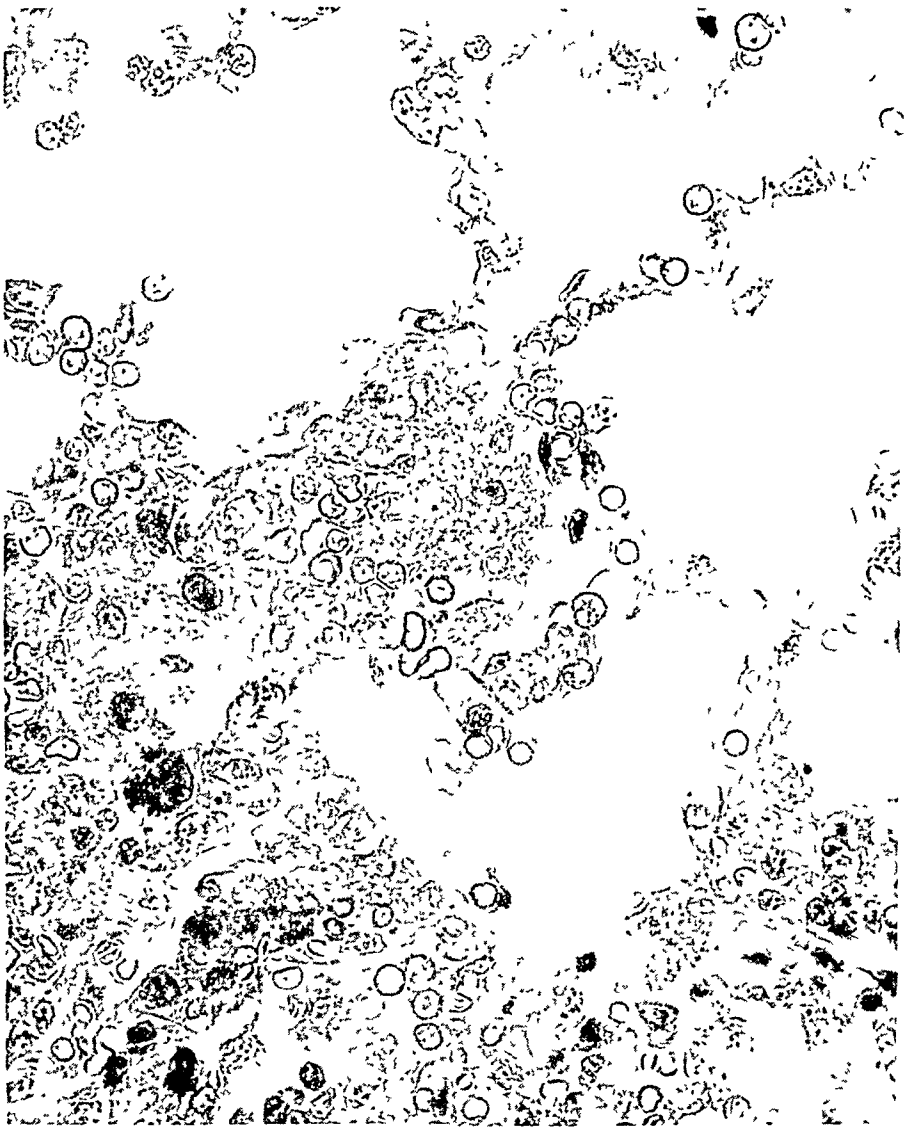


Fig. 5.—One detail of the "parenchymatous alveolitis" demonstrated in the previous figure, as seen under lenses of high power. The cells lining the walls of the alveoli, and not others, have given birth to the intra-alveolar "epithelioid" cells. The reaction is "proliferative" because of the fact that a proliferation of the "epithelial" cells has occurred. The cells adjacent to the "tubercle" should be noticed. Methylene blue and eosin;  $\times 600$ .

histologic picture which is shown in figures 4 and 5, was as follows: The focal cellular agglomerations noticed immediately after the infection had increased in number and in size. They occupied several alveoli, the confines of which could still be made out. The cells filled the entire lumen of each air sac almost to

capacity. The cells were mostly of the large variety described; their nuclei were irregularly round, with coarse chromatin particles, and the cytoplasm was foamy with "ragged" outlines, containing, in some instances, tubercle bacilli. The agglomeration of the cells, which had all the marks of epithelioid cells, gave to these foci the appearance of "primitive tubercles."

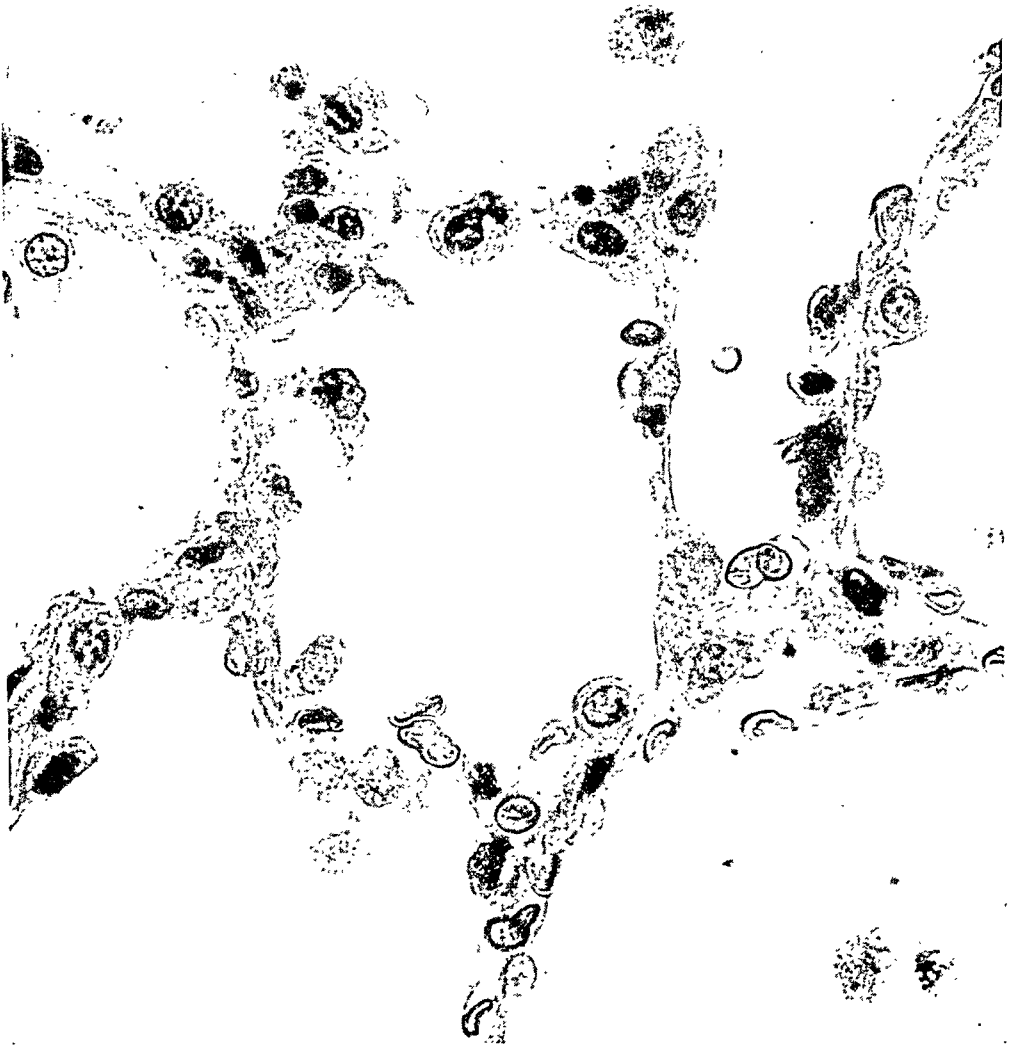


Fig. 6.—A section from an air sac near a tubercle found in the lung of an animal that died ninety hours after intratracheal infection. Note the topography and the morphology of the "resting" and of the "wandering" cells. Methylene blue and eosin;  $\times 850$ .

Here, too, a thorough investigation of the tissue revealed that the endothelium of the septal capillaries remained passive; their lumina were but slightly filled with red cells.

It was obvious that the intra-alveolar exudate resulted from the cells lining the wall of the air sac, which had instantly undergone the changes just recorded. This, moreover, could be ascertained from the inspection of the sections at the border of the "primitive tubercle," which revealed the following: The tubercle

was rather sharply circumscribed. With the lenses of lower power one noticed merely an increase in the number of "nuclei" per microscopic field. But with the oil immersion lenses the topography and also the morphology of these cells could clearly be made out (fig. 6). They lay, as already said, outside on the wall of the respiratory vesicle facing the alveolar lumen. They were polymorphic, varying from a somewhat elongated endothelial-like form to a monocytoïd variety; they occurred singly or in groups of two or three, and they showed morphologic transitions, as did the cells seen about one minute after the infection. They were more conspicuous at the intersection of the capillaries.



Fig. 7.—A "primitive tubercle" found in a rabbit's lung ten minutes after the intratracheal injection of bovine tubercle bacilli. Here, too, there occurred a "parenchymatous alveolitis" resulting from the proliferated "alveolar epithelial" cells. Methylene blue and eosin;  $\times 850$ .

At this period one noticed more granulocytes and also a few pseudo-eosinophilic cells. There also were conspicuous proliferations of cells around the thin-walled veins.

There was no serum or blood in the alveoli, and the lymph follicles maintained their passive attitude. Here, too, no traces of a continuous "epithelial membrane" could be found.

*After Ten Minutes.*—A section from a lung removed ten minutes after the intratracheal infection is seen in figure 7. From this photomicrograph again it will be seen that the "tubercle" is intra-alveolar (parenchymatous alveolitis);

that it is made up of large epithelioid cells, and that the cells forming the tubercle and those lying close to the septum are identical as to the finest details.

*After One Week.*—The reaction increased in intensity every few minutes, the difference between the earliest response of the pulmonary tissues and that in the later periods being no more than quantitative.

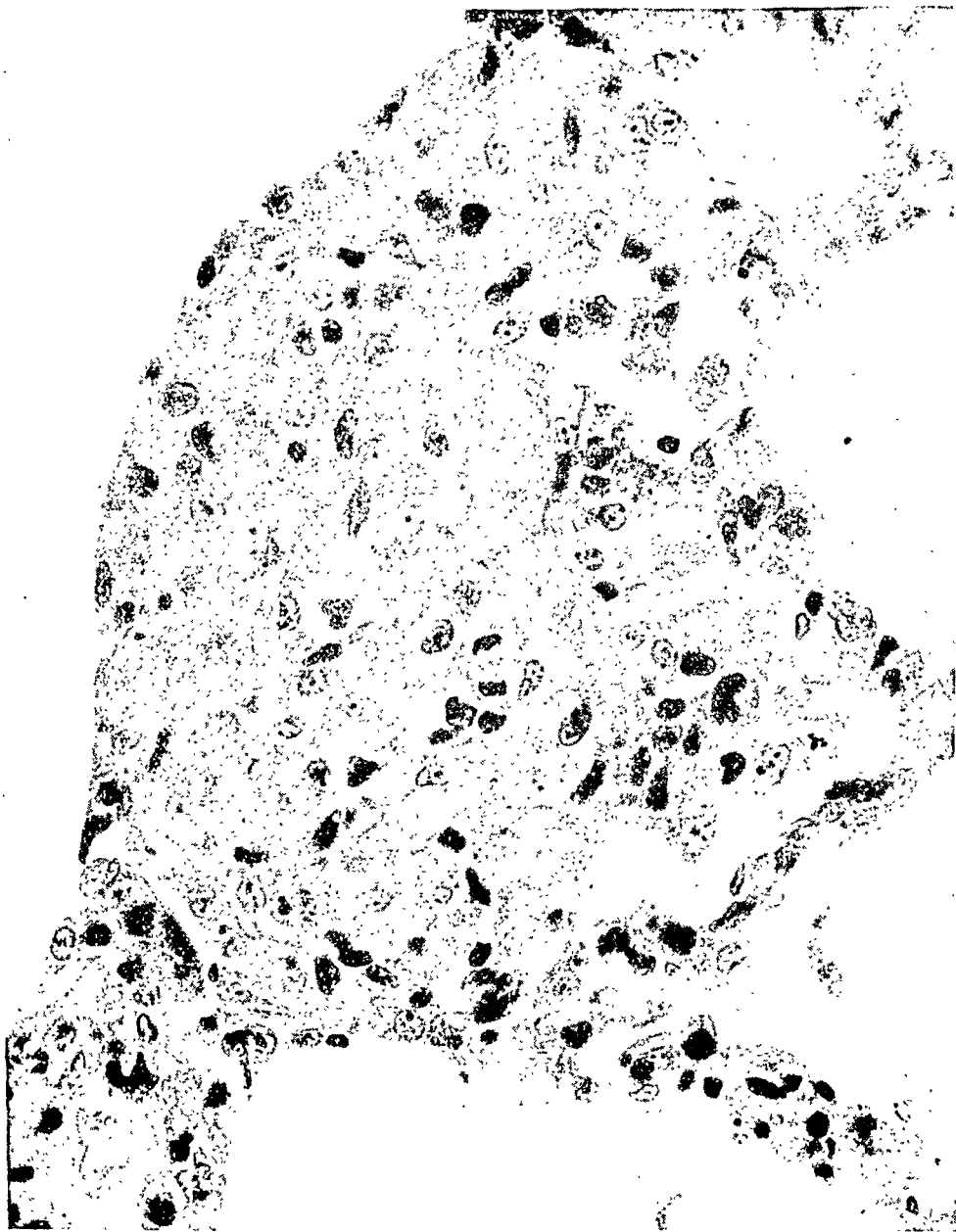


Fig. 8.—A "metastatic" miliary tubercle found in the lung of a rabbit that had been infected seven days previously. A cell in mitosis is seen in the center of the figure. Methylene blue and eosin;  $\times 600$ .

With the spread of the disease, primitive tubercles united to form large areas of tuberculous granulation tissue, the centers of which were undergoing caseation. This, under the conditions of the present experiments, occurred at the fourth or

at the fifth day after infection. But even at this stage, the histogenesis of the primitive tubercle could be traced with reasonable certainty, provided the edge of a "granuloma" was examined, or if the investigation was directed toward areas where new "metastatic" tubercles were formed as a result of the dissemination of the acid-fast bacilli throughout the lung.

As an illustration, the structure of the tuberculous tissue seven days after the intratracheal injection of Koch's bacillus may be studied. This tissue is represented in figures 8, 9 and 10.

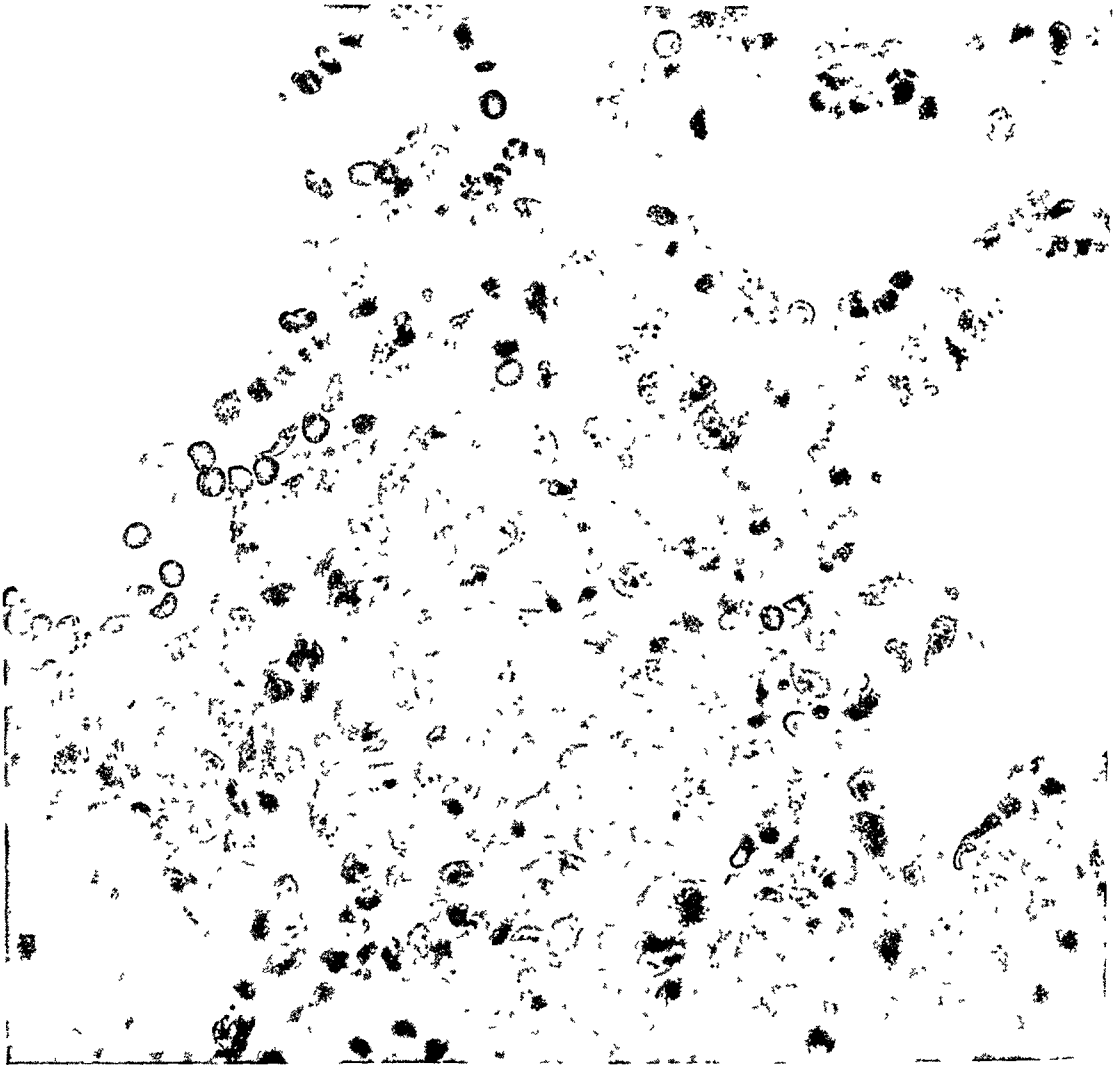


Fig. 9.—A 7 day old intra-alveolar tubercle. Note the cells lining the septum and compare them with those forming the "exudate." A few granulocytes are seen here and there. Methylene blue and eosin;  $\times 600$ .

The tubercles illustrated in figures 8 and 9 were found in the lungs of two different animals. In the aerogenous infection 1 week old, the "metastatic" tubercle was a counterpart of what one had observed in the lungs five minutes after infection. The "alveolar epithelial" cells, and no others, evolved into typical epithelioid cells, which agglomerated to form the miliary tubercle. The morphology of the "epithelioid" cells is particularly demonstrated in figure 10. This

section, too, was made from the lung of a rabbit killed seven days after the intratracheal infection. The last three photomicrographs show, then, tuberculous lesions of the same age, yet these lesions reveal striking differences in their appearance, which indeed is characteristic of the "polyblastic" nature of the "alveolar epithelium."

There still exists a discussion among phthisiologists as to whether the primitive tubercle originates in the lumen of the alveolus or is formed within the connective



Fig. 10.—The histologic appearance of the tubercular infection of seven days' duration. The exquisitely typical "epithelioid" cells give the impression that they are "streaming" from the septum toward the infected alveolar lumen. Hematoxylin and eosin;  $\times 600$ .

tissue of the septum. The experiments presented here have shown that the primitive tubercle is essentially an intra-alveolar structure, or what I should designate as a "parenchymatous alveolitis" (figs. 4, 7, 8 and 9). If one is to use the terminology of some German writers, the reaction is a proliferative one, because of the fact that there occurs a proliferation of the "alveolar epithelial" cells.

*After Thirteen Days.*—The border of the “granuloma” was of interest in still another way: The left segment in figure 11 shows a lesion of thirteen days’ duration. From this picture it will be seen that the necrotic (caseated) center is surrounded by a rim of healthy cells. It is interesting that, at the border, the conglomerate tubercle was formed not only as a result of a proliferation of its own elements, but also through “conversion” of those “resting” on the alveolar wall.

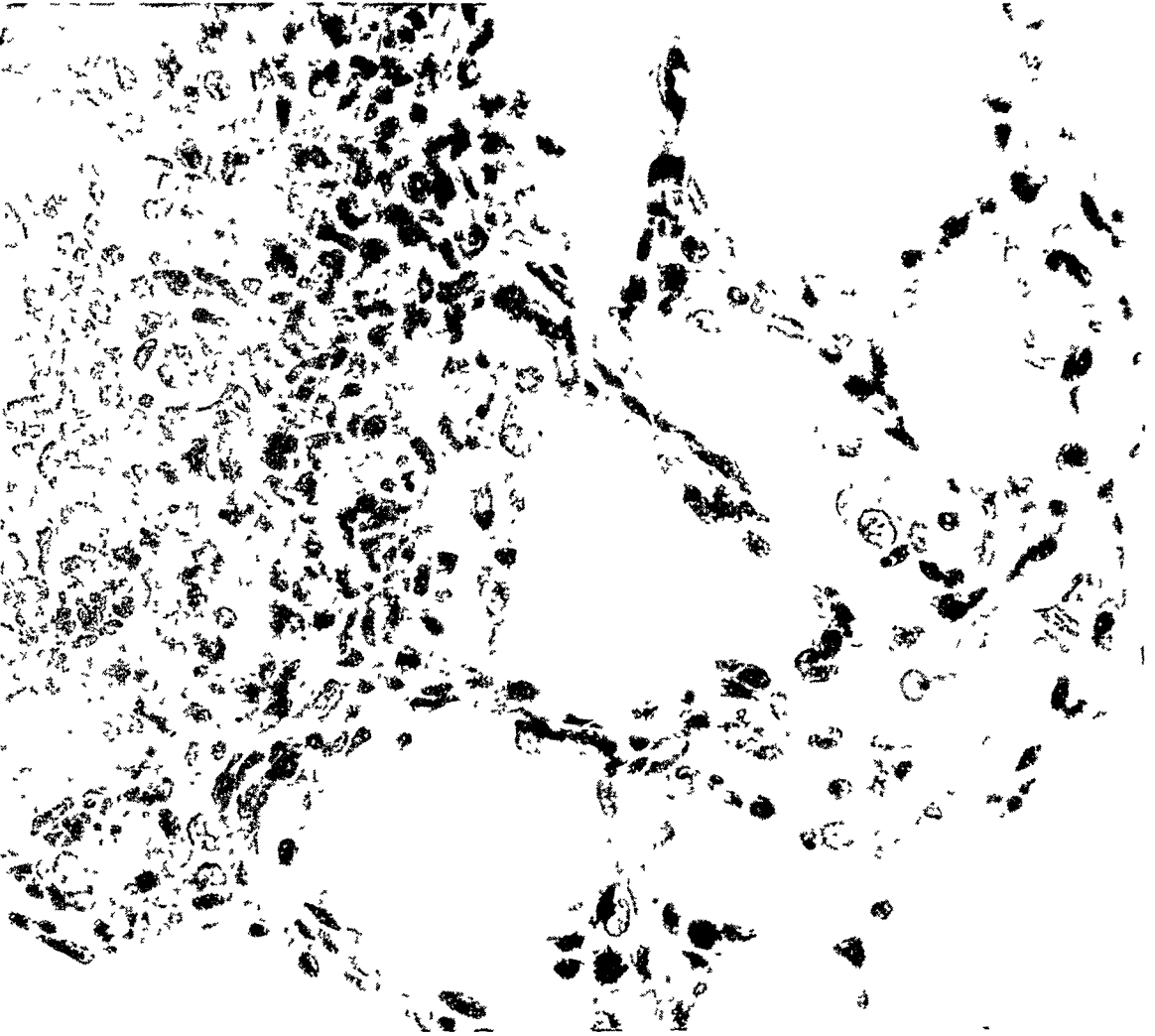


Fig. 11.—The picture as seen at the border of a tubercular granuloma of thirteen days’ duration. The “conversion” of the adjacent “resting” cells and their “streaming” toward the aging focus are remarkable. Hematoxylin and eosin;  $\times 600$ .

The formation of giant cells occurred within a few hours after the infection by way of fusion of “alveolar epithelial” cells and also by nuclear division of these cells.

In brief, then, at all stages of the tuberculous infection the essential response to the acid-fast bacilli in the respiratory alveolus was that of the cells “lining” the wall of the air sac, commonly designated as “respiratory epithelium.”

When tubercle bacilli reached the peribronchial lymphoid tissue, they inaugurated a tubercle in the “clear zone” of the reticular cells, which evolved into

epithelioid cells. As a result of the proliferation of these cells the wide zone of the small round cells became thinner. However, a transformation of the lymphoid cells into macrophages and epithelioid cells could not be observed.

The perivascular and peribronchial mesenchymal cells, too, underwent morphologic changes when tubercle bacilli settled adjacent to them. They, moreover, proliferated, forming perivascular collars of macrophages. Perivascular agglomerations of thick layers of small round cells could not be seen in the lungs of the tuberculous rabbits, like those found in the "model infections" of rabbits or of cats with oils <sup>4</sup> (fig. 12).

In the bronchi progressive changes were seen in the so-called basal cells of the bronchial mucosa, evidenced by focal proliferation of the cells and also by occa-

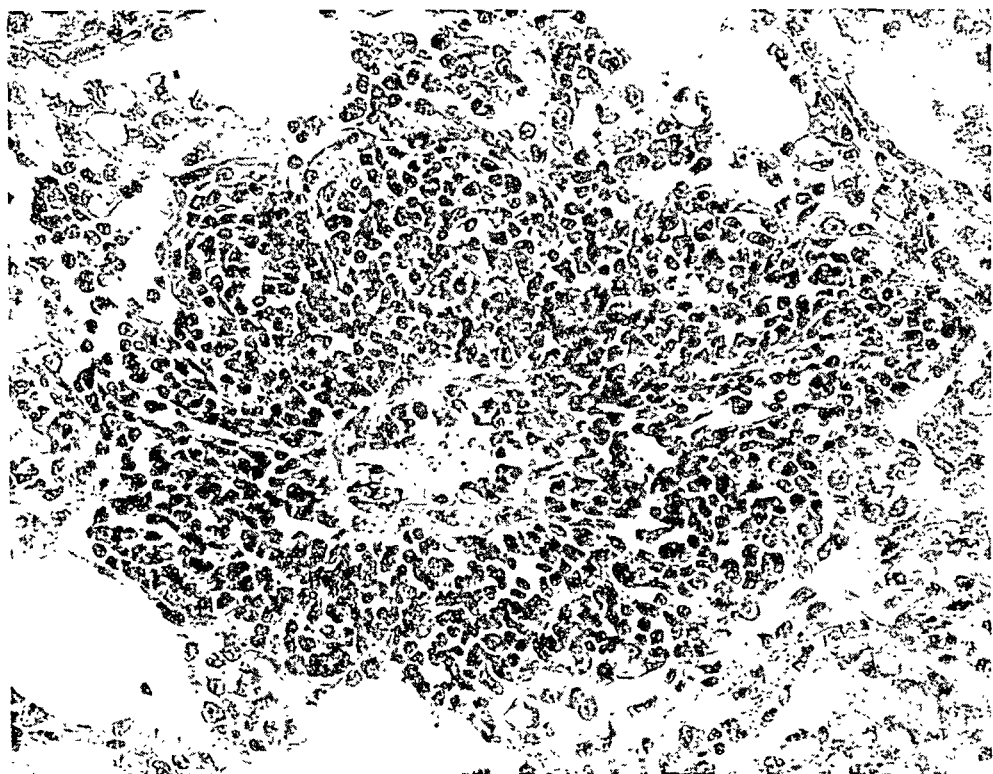


Fig. 12.—Perivascular proliferation of lymphoid cells in the lung of a cat that had received one injection of oil by way of the trachea. Hematoxylin and eosin;  $\times 300$ .

sional mitoses. The columnar cells revealed early regressive changes ending in complete degeneration near tuberculous granulation tissue. The notion of pathologists of the past century (recently revived by Duthie<sup>5</sup>) that the bronchial epithelium is actively phagocytic for bacteria, being a source of the "dust cells," was not borne out by these experiments. I have observed that the alveolar wandering macrophages ("dust" cells) often "invade" the bronchial lumen and wall as well, this certainly leading to the erroneous impression of the transformation of the columnar bronchial epithelium into "dust" cells.

4. Fried (footnote 1, second reference).

5. Duthie, E. S.: Phagocytosis by Bronchial Epithelium in the Lungs of Mice, *J. Path. & Bact.* **33**:547, 1930.



## COMMENT

The histogenesis of the tubercle in the lung is as debatable today as it was decades ago. One is still uncertain not only of the strain, but also of the source of the cells of which the primitive pulmonary tubercle is made up.

The discussion has become particularly acute since the inauguration of the teachings of Metchnikoff on inflammation. Baumgarten,<sup>6</sup> who was the first to study experimental tuberculosis with the then discovered tubercle bacillus is often quoted as being the original author of the theory whereby the fixed tissue cells were said to be responsible for the tubercle, while Metchnikoff<sup>7</sup> is referred to as the partisan of the hematogenous origin of the pulmonary epithelioid cell. In reality the "fixed cell" theory was the one that prevailed among workers of the past.

The conception of Virchow that the tuberculous granulation tissue develops only from the connective tissue did not receive general recognition by his contemporaries. Reports by numerous workers were to the effect that every cell in the organism took part in the formation of the tubercle. A French writer of the seventies of the past century, who gave a good summary of the question, wrote: "The tuberculous granulation tissue has the sad privilege to be developed at the expense of the cells of the connective tissue, of the epithelium of the serous membrane in the lungs, of the hepatic cells, of the renal cells, and of the smooth muscle fibers" (Thaon,<sup>8</sup> Grancher<sup>9</sup>). In Germany similar views were simultaneously expressed in a detailed study on tuberculosis by Arnold.<sup>10</sup>

The results obtained by Baumgarten<sup>6</sup> from his experimental studies with Koch's bacillus did not differ from those of his predecessors. He, too, traced the origin of the tubercle to the endothelial cells, to the hepatic and biliary epithelial cells, to the renal epithelium and also to the bronchial and the respiratory (alveolar) epithelium.

The early investigations by Metchnikoff<sup>11</sup> revealed, however, that the phagocytosis of the tubercle bacillus is the essential function of

6. Baumgarten, P.: Experimentelle und pathologisch-anatomische Untersuchungen über Tuberkulose, *Ztschr. f. klin. Med.* **9**:93 and 243, 1885; **10**:24, 1886.

7. Metchnikoff, E.: *Leçons sur la pathologie comparée de l'inflammation*, Paris, Masson & Cie, 1892.

8. Thaon, L.: *Recherches sur l'anatomie pathologique de la tuberculose*, Paris, Duval, 1873.

9. Grancher, J.: *De l'unité de la phtisie*, Thèse de Paris, 1873; *Tuberculose pulmonaire*, *Arch. de physiol. norm. et path.* **10**:2, 1878.

10. Arnold, Julius: *Beiträge zur Anatomie des miliären Tuberkels*: IV. Ueber disseminierte Miliartuberkulose der Lungen, *Virchows Arch. f. path. Anat.* **88**: 397, 1882.

11. Metchnikoff, E.: Ueber die phagocytäre Rolle die Tuberkelriesenzellen, *Virchows Arch. f. path. Anat.* **113**:63, 1888; footnote 7.

the macrophage. He therefore emphasized the fact that: "le tubercle est composé d'une réunion de phagocytes d'origine mésodermique qui affluent vers les endroits où se trouvent les bacilles et les englobent." ("The tubercle is made up of a gathering of phagocytes of mesodermic origin, which flock toward the areas where the bacilli are located and engulf them.")

Although this conception gained ground, it was still believed that in pathologic conditions of the lungs the alveolar epithelium was the essential active element. An investigation by Arnold<sup>12</sup> on pneumoconiosis showed to him that the earliest and most constant effect of the inhaled dust was conspicuous in the alveolar epithelium. Ribbert (cf. Fleck<sup>13</sup> and Laehr<sup>14</sup>), by infecting animals with streptococci and staphylococci, respectively, noted that the micro-organisms were actively phagocytosed by the respiratory epithelium. (This served as an argument against the conception that active phagocytosis is the monopoly of the "so genannte Makrophagen" [Ribbert]).

Studies on pneumonia in man have likewise revealed the readiness with which the "alveolar epithelial" cells respond in acute inflammatory conditions of the lungs. Of particular interest were the observations made on the so-called "pneumonia desquamativa" which was for the first time reported by Buhl.<sup>15</sup> The disease was said to begin with morphologic changes in the alveolar epithelium, with proliferation of these cells, and then "desquamation," forming the intra-alveolar exudate. In the obliterating forms of this disease (pneumonia desquamativa obliterans) the reticulum, too, was claimed to be formed by the respiratory epithelium (Galdi<sup>16</sup>). Cornil,<sup>17</sup> who, with many others, regarded the endothelium of blood and lymph channels as being the essential element in the field of inflammation, compared the function of the respiratory epithelium to that of the lining of the blood vessels. He wrote, for instance "The phenomenon of beginning inflammation is essentially the same whether it concerns the connective tissue or the lungs."

Apparently the "endothelial" theory of inflammation began to prevail, for in his lectures on inflammation Metchnikoff<sup>7</sup> stated that "the cells

12. Arnold, Julius: Staubinhalation und Staubmetastase, Leipzig, F. C. W. Vogel, 1885.

13. Fleck: Die acute Entzündungen der Lunge, Dissertation, Bonn, 1886.

14. Laehr, G.: Ueber den Untergang des Staphylococcus pyogenes aureus in den durch ihn herforgerufenen Entzündungsprozessen, Dissertation, Bonn, 1887.

15. Buhl, L.: Lungenentzündung, Tuberkulose und Schwindsucht. Zwölf Briefe an einen Freund, ed. 2, Munich, R. Oldenbourg, 1873.

16. Galdi, F.: Ueber einige von den gewöhnlichen abweichende Pneumonie-Formen, Deutsches Arch. f. klin. Med. **75**:239, 1903.

17. Cornil, V.: De modifications que subissent les cellules endotheliales dans les inflammations, Arch. de méd. expér. et d'anat. path. **9**:9, 1897.

in tuberculosis (the epithelioid as well as the giant cells) are made up exclusively of phagocytic elements; that is, of the large mononuclear leukocytes and of the stellate Kupffer cells which are of endothelial origin; in the spleen and the lymph nodes the tubercle results from a collection of the large mononuclear phagocytes *of these organs*, and in the lungs it is formed at the expense of the endothelial cells of the blood vessels with the assistance of the leukocytes." Metchnikoff did not mention the origin of the pulmonary "leukocytes" until a few years later when he <sup>18</sup> wrote: "For long the large dust cells of the respiratory channels were looked on as being epithelial cells that were capable of taking up carbon particles, micro-organisms and other foreign bodies. In reality these elements are nothing more than white corpuscles that have immigrated into the alveoli and bronchi."

The apparent inconsistency of Metchnikoff in regard to the inflammatory reactions in tuberculosis of the lungs undoubtedly resulted from the fact that the conception of the fixed macrophages (histiocytes) was not clearly understood by pathologists of that period. The clasmatoocyte described by Ranvier <sup>19</sup> was thought to be of hematogenous origin, being present in the taches laiteuses of the omentum only, and their virtual significance as well as their relation to the histiocytic elements found elsewhere in the body was not appreciated.

More recent studies on mesenchymal reactions in tuberculosis of organs other than the lungs have shown that the primary reaction to Koch's bacillus is essentially that of the local elements (Buday;<sup>20</sup> Joest and Emshoff;<sup>21</sup> Evans, Winternitz and Bowman;<sup>22</sup> Krause <sup>23</sup>). The question of the source of the "exudate" in inflammatory and congestive conditions of the lungs, and particularly of the cells that form the pulmonary tubercle, has remained controversial.

The experiments presented here have shown, first, that in tuberculosis of the lung, too, the cells that primarily respond to Koch's bacillus are not called from outside sources, but are produced in situ. In the second place, they have shown that the source of these cells is not the

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18. Metchnikoff, E.: *L'immunité dans les maladies infectieuses*, Paris, Masson & Cie, 1901.

19. Ranvier, L.: *Des clasmatoocytes*, Arch. d'anat. micr. **3**:123, 1900.

20. Buday, K.: *Experimentelle histologische Studien über die Genese der Miliartuberkels*, Virchows Arch. f. path. Anat. **186**:145, 1906.

21. Joest, E., and Emshoff, E.: *Studien über die histogenese des Lymphdrüsentuberkels und die Frühstadien der Lymphdrüsentuberkulose*, Virchows Arch. f. path. Anat. **210**:188, 1912.

22. Evans; Winternitz, and Bowman: *An Experimental Study of the Histogenesis of the Miliary Tubercle in Vitrally Stained Rabbits*, J. Exper. Med. **19**:15, 1914.

23. Krause, A. K.: *The Anatomical Structure of the Tubercle from Histogenesis to Cavity*, Am. Rev. Tuberc. **4**:137, 1927.

endothelium of the pulmonary capillaries (endothelial leukocytes), but cells that "line" the walls of the air sacs, as well as those that are normally present in the loose connective tissue of the septums.

Let me analyze my observations: When the tubercle bacilli reached the air sac, the reaction caused by them was instantaneous. For within one minute after the aerogenous infection (figs. 1, 2 and 3), there occurred a marked proliferation, as well as morphologic changes, of cells lying in and on the septums. These cells, which are normally barely visible, giving to the air sac a "naked" appearance, revealed characteristics that are proper to fixed or to wandering macrophages (monocytes and clasmotocytes). A number of these cells lay free in the alveolar lumen, showing phagocytosis of the tubercle bacilli; the bacilli also were found in the cytoplasm of some of the enlarged sessile cells. Cells in mitosis and in amitosis were seen here and there, and within the next few minutes primitive tubercles made up of these (epithelial) cells transformed into epithelioid cells were seen scattered over the sections. At this period, too, outlines of giant cells could be made out budding from the septums, resulting from the "agglutination" of individual cells and also from the nuclear division of cells.

As a rule the newly formed cells proliferated inside the lumen of the alveolus, causing a "parenchymatous alveolitis." This process ordinarily involved a group of several alveoli, of which the smaller were completely occluded by the epithelioid cells, while the large were occupied only in part.

It is remarkable, then, that in addition to the prompt morphologic changes and the rapid proliferation, the sessile and the free cells also displayed an instantaneous phagocytosis of the acid-fast bacilli.

The endothelium of the capillaries of the septums showed no particular changes. Their lumina contained a moderate number of red cells, but no mononuclear leukocytic elements.

Particularly instructive, too, were the pictures in close proximity to the primitive tubercle. It appeared that not only did the morbid process advance by proliferation of its own elements, but at the borders it also "converted" the adjacent cells. Here, also, the cells while still "resting" on the septums, had taken on new morphologic aspects, thus giving them the appearance of large monocytes (fig. 13). In proximity to the primitive tubercle these cells could be seen to "stream" singly or in groups to join and to enforce the already formed "miliary granule." Thus, when the agglomerated tubercle had reached considerable dimensions, and its center had begun to degenerate (caseation), its periphery was made up of new elements that resulted not only from proliferation of its own cells, but also from the mobilization of those resting in the adjacent areas (figs. 11 and 13).

As I have said, the reaction was confined to the cells lining the septums and to those found within the septums as well. The wall of the air sac, representing, indeed, no more than a "spider's web" of loose connective tissue enforced with a few elastic fibers, also contains interlacing bundles of innumerable capillaries and a sparse number of scattered cells. These cells, when sectioned at various angles, appear either as elongated cells lying "astride" on the capillary, as do the

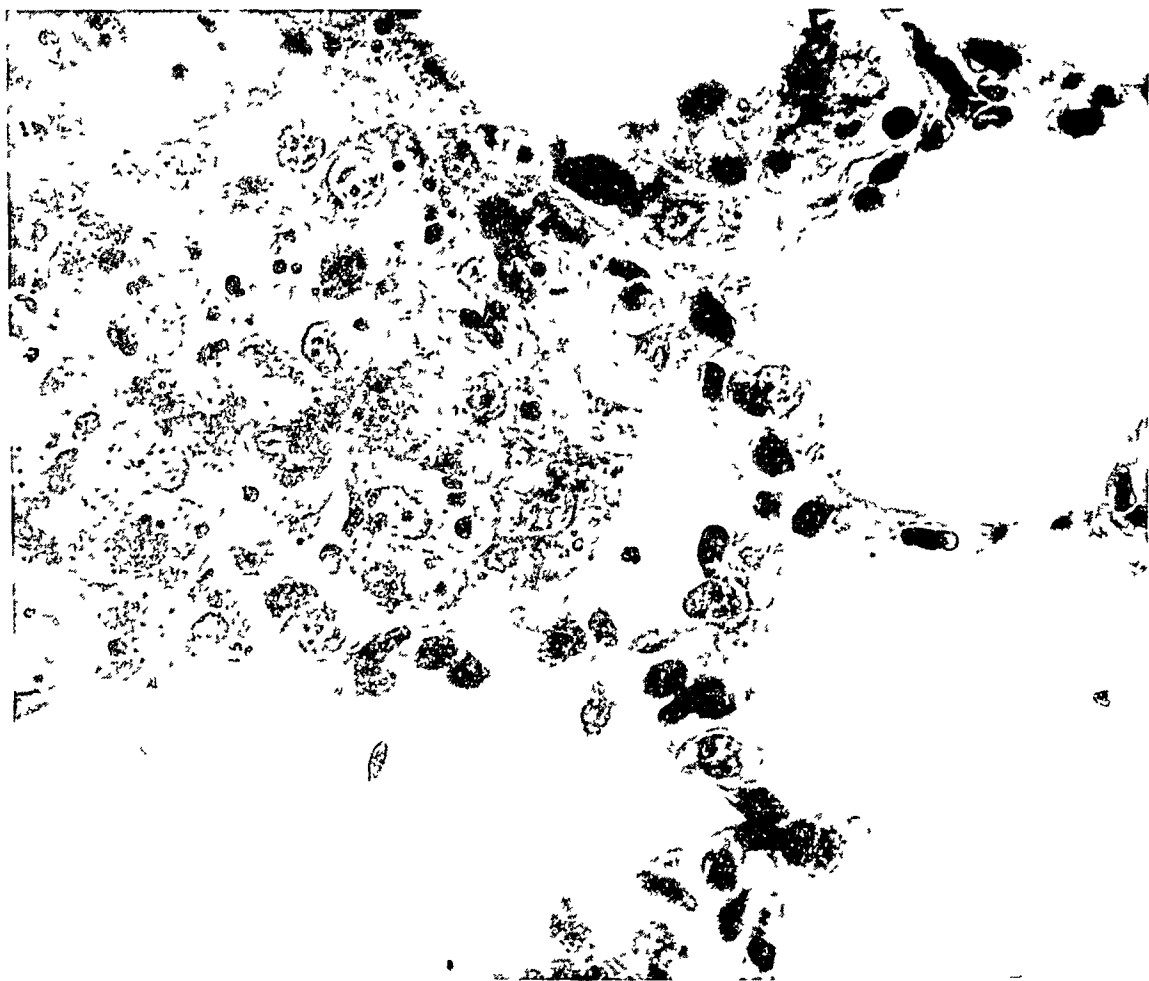


Fig 13—Note the cells "lining" the walls of the air sacs at the frontier of an advancing tubercle. Compare these cells with those that had formed the "miliary granule" seen on the left side of the picture. Methylene blue and eosin;  $\times 800$

"adventitial" cells of Marchand, or as round lymphocytoid cells having somewhat bean-shaped nuclei with coarse, darkly stained chromatin particles. The resemblance of these cells to lymphocytes is remarkable, and was probably responsible for the opinion expressed by Metchnikoff that the lymphocytes are to a large degree the source of the epithelioid cells. "Si les lymphocytes même," wrote Metchnikoff, "ne sont encore point des phagocytes, ils le deviennent bientôt, après s'être transformé

en cellules epithelioides." ("If the lymphocytes," wrote Metchnikoff, "are not yet phagocytes, they soon become so after having been transformed into epithelioid cells.") This property of the small round cell was also stressed by Maximow,<sup>24</sup> who was, however, of the opinion that "the small lymphocytes, as a rule, first have to enter the blood and to circulate there before being activated for the progressive development under the influence of suitable external conditions." Bloom<sup>25</sup> produced convincing experimental evidences of such a metamorphosis in vitro, but Ferrata<sup>26</sup> and Naegeli<sup>27</sup> were opposed to this view.

In the experiments presented here, the pulmonary lymphocytes did not seem to play a rôle in the provision of macrophages. For even in the peribronchial lymphoid accumulations the tubercle developed in the midst of the "clear zone" of reticular cells, which gradually had evolved into epithelioid cells, but no transitions could be seen between these cells and the small round cells. Likewise the fully developed tubercle in these cases was composed of a central giant cell surrounded by epithelioid cells with a very scant number of "small round cells," unlike the picture one ordinarily sees in tubercles found in protracted cases of human tuberculosis.

Apparently the cells found in the walls of the septums are not other than undifferentiated mesenchymal cells, which here, as indeed elsewhere in the organism, readily serve as a source of the wandering macrophage.

The topography of these small lymphocytoid cells about the capillaries of the air sacs imitates the same condition found throughout the body, and the suggestion was made in a recent textbook of histology<sup>28</sup> that they are probably nothing other than adventitial cells.

That the adventitia of blood channels is accompanied by peculiar cells was for the first time demonstrated by Rouget,<sup>29</sup> who believed them to be "muscle cells" (cellules de Rouget) playing a rôle in the

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24. Maximow, A.: *Handbuch der microscopischen Anatomie des Menschen*, Berlin, Julius Springer, 1927, vol. 1, p. 2.

25. Bloom, W.: Ueber die Verwandlung der Lymphocyten der Lymphe der Ductus thoracicus der Kaninchens in Polyblasten (Makrophagen) in Gewebeskulturen, *Centralbl. f. allg. Path. u. path. Anat.* **39**:3, 1927.

26. Ferrata, A., quoted by Maximow, A.: *The Lymphocytes and Plasma Cells*, *Special Cytology*, edited by E. V. Cowdry, New York, Paul B. Hoeber, 1928, vol. 1, p. 321.

27. Naegeli, O.: *Blutkrankheiten und Blutdiagnostik*, ed. 4, Berlin, Julius Springer, 1923.

28. Maximow, A.: *A Text-Book of Histology*, Philadelphia, W. B. Saunders Company, 1931.

29. Rouget, C.: *Mémoire sur le development, la structure et les propriétés physiologiques des capillaires sanguins et lymphatiques*, *Arch. de physiol. norm. et path.* **5**:603 and 639, 1873.

contraction of the capillaries. Eberth<sup>30</sup> observed "perithelial" cells around the blood vessels of the central nervous system, which he thought were epithelial. ("Peritheliomas" were believed to originate from these cells.) Marchand<sup>31</sup> found them to be an essential part of the vascular adventitia (adventitial cells of Marchand). Goldmann<sup>32</sup> convincingly demonstrated that these cells belong to the reticulo-endothelial (macrophage) system. Krogh<sup>33</sup> and his pupil Vimtrup<sup>34</sup> confirmed the observations of their predecessors, and a Swiss histologist, Zimmermann,<sup>35</sup> designated them as pericytes (figs. 14 and 15).

Whatever the nomenclature of these cells may be, they are, it is reasonably certain, of mesenchymal origin, and act as such: they store dyes and oils; they avidly phagocytose micro-organisms; they play a

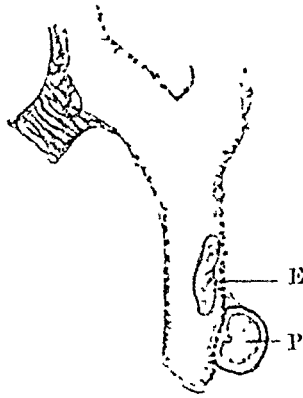


Fig. 14.—A capillary from the thymus: *E*, endothelium; *P*, pericyte. (After Plenck; copied by Benninghoff, A.: *Blutgefässe und Herz*, in Möllendorff: *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1925, vol. 6, p. 30).

rôle in the extrahepatic formation of bile;<sup>36</sup> they likewise participate in the formation of the tubercle, as seen in the experiments presented here.

30. Eberth, C. J.: Zur Entwicklung des Epitheliomas (Cholesteatomas) der Pia und der Meningen, *Virchows Arch. f. path. Anat.* **49**:48, 1870.

31. Marchand, F., in Krehl and Marchand: *Handbuch der allgemeinen Pathologie*, Leipzig, S. Hirzel, 1924, vol. 4.

32. Goldmann, E. A.: Die äussere und innere Secretion des gesunden und kranken Organismus im Lichte der "vitalen Färbung", *Beitr. z. klin. Chir.* **64**:192, 1900; **78**:1, 1912.

33. Krogh, A.: *Anatomy and Physiology of Capillaries*, New Haven, Conn., Yale University Press, 1928.

34. Vimtrup, B.: Beitrag zur Anatomie der Capillären, *Ztschr. f. Anat. u. Entwicklungsgesch.* **65**:150, 1922.

35. Zimmermann, K.: Der feinere Bau der Blutcapillären, *Ztschr. f. Anat. u. Entwicklungsgesch.* **68**:29, 1923.

36. Fried, B. M.: The Rôle of the "Alveolar Epithelium" in the Extrahepatic Formation of Bile, unpublished data.

The "defensive" rôle of the lung is obvious not only from the instantaneous response of its local (alveolar wall) macrophages, but from the natural history of the aerogenous infections with Koch's bacillus. Thus, under the conditions of the experiments discussed in this article, rabbits that have been infected with the bovine type of tubercle bacilli by way of the trachea survive three and more times

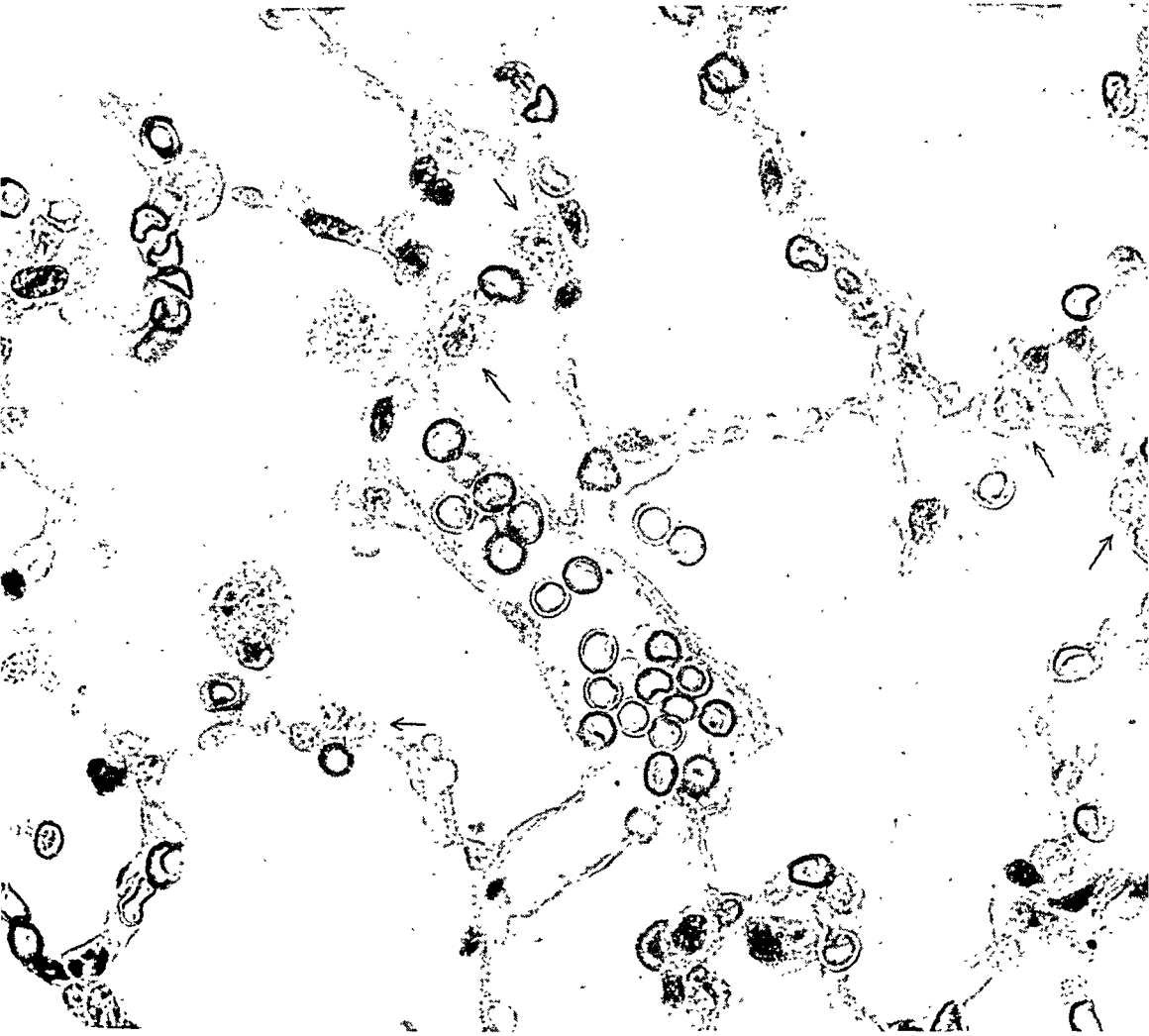


Fig. 15.—An area of a rabbit's lung remote from tuberculous granulation tissue. The cells around the capillaries should be noticed. Compare their topography with that of the pericyte (*P*) shown in figure 14. Methylene blue and eosin;  $\times 850$ .

longer than those that have received a similar dose of bacilli by way of the blood stream. It also is interesting that in many animals that have been infected via the trachea the acid-fast micro-organisms apparently remain "immured" in the lung "for life," for in these instances the micro-organisms cannot be detected outside these organs. The lungs,



then, are not only a "sieve" or a "filter" (Spanier <sup>37</sup>), but are a virtual "fortress" which retains and destroys not only foreign elements, but pathogenic micro-organisms (staphylococci, anthrax bacilli, pneumococci).

It is true that hematogenous elements (monocytes and also lymphocytes) contribute to the formation of the tuberculous granulation tissue of the lung. However, the afflux of these cells from outside sources occurs at a later date and obviously does not play an important rôle when infection occurs by way of the respiratory tract.

It is of interest that even granulocytes, which are found in numbers in the lungs of rabbits infected with tubercle bacilli by way of the blood stream, are insignificant in aerogenous infections. The rapid appearance of these cells in the lungs after infection was thought by Töppich <sup>38</sup> to be the result of their local formation from the "younger mesenchymal cells." In reality the granulocytes reach the lung from the periphery.

Studies on the peripheral blood of animals infected with tubercle bacilli <sup>39</sup> reveal that the infection is followed by an almost instantaneous leukopenia. The granulocytes, then, that have disappeared from the circulation, are found in the visceral organs and particularly in the lungs. They often contain engulfed tubercle bacilli, and in their turn are phagocytosed by the local macrophages. (This will be discussed in detail in a report on hematogenous infection with tubercle bacilli.)

That the lungs react as do mesenchymal organs, and that the essential source of the reactive elements is not the blood or the endothelium of the capillaries, but the cells lining the air sacs, as well as those found within the septums, is apparently, not contested any more. What one disputes today is the "alveolar epithelium," the mesenchymal nature of which is denied on the ground that it is in conflict with the "classic" descriptions of the embryology of the lung. However, recent investigations of the fetal lung revealed that there exists a great uncertainty as to the embryology of the primordium of the air sacs. Thus Rose <sup>40</sup> produced evidences to show that the lung is developed from two different germ layers, endoderm for the bronchi and mesoderm for the "parenchyma." Bloom, in Maximow's <sup>28</sup> textbook of histology, was as yet undecided, urging "a thorough embryological

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37. Spanier, Philipp: Ueber die Bedeutung der Lungen im Stofftransport innerhalb der Organismus, Eine experimentelle Untersuchung, Beitr. z. Klin. d. Tuberk. **76**:507, 1931.

38. Töppich, G.: Die Zellulären Abwehrvorgänge in der Lunge bei Erst und Wiederinfektion mit Tuberkelbazillen, Krankheitsforschung **9**:15, 1925.

39. Fried, B. M., and Dameshek, William: The Picture of the Peripheral Blood in Rabbits Infected with the Tubercle Bacillus by Way of the Trachea, unpublished data; Experimental Agranulocytosis, Arch. Int. Med., to be published.

40. Rose, S. B.: The Finer Structure of the Lung, Arch. Path. **6**:36, 1928.

investigation of the lungs in the later stages of intra-uterine life," while Policard <sup>41</sup> and Chiodi <sup>42</sup> believed that originally there is a cuboidal epithelium lining the air sacs, but that it disappears in the later months of fetal life to be replaced by mesenchymal cells.

In fact the histologists' description to the effect that in the adult lung there is a transitional epithelium between the bronchiolic epithelium and that of the supposed cuboidal alveolar epithelium was not supported by



Fig. 16.—A segment of a respiratory bronchiole to show that there is no transitional epithelium between the bronchiolar epithelium and the alleged alveolar epithelium. The abrupt ending of columnar cells that line the bronchiole is indicated by an arrow. Methylene blue and eosin;  $\times 600$ .

41. Policard, A.: Les nouvelles idées sur la disposition de la surface respiratoire, *Presse méd.* **37**:1243, 1929.

42. Chiodi, V.: Sulla natura delle cellule libere del pulmone e del rivestimento dell'alveolo pulmonare, *Arch. d'anat., d'histol. et d'embryol.* **8**:313, 1928.

these studies. From figure 16 it may be seen that the epithelium of the bronchiole ends abruptly, and is "followed" by the "naked" walls of the air sacs (fig. 16).

The assertion of the epithelial nature of the "alveolar epithelium" was also based on the observation that these elements, which are incon-

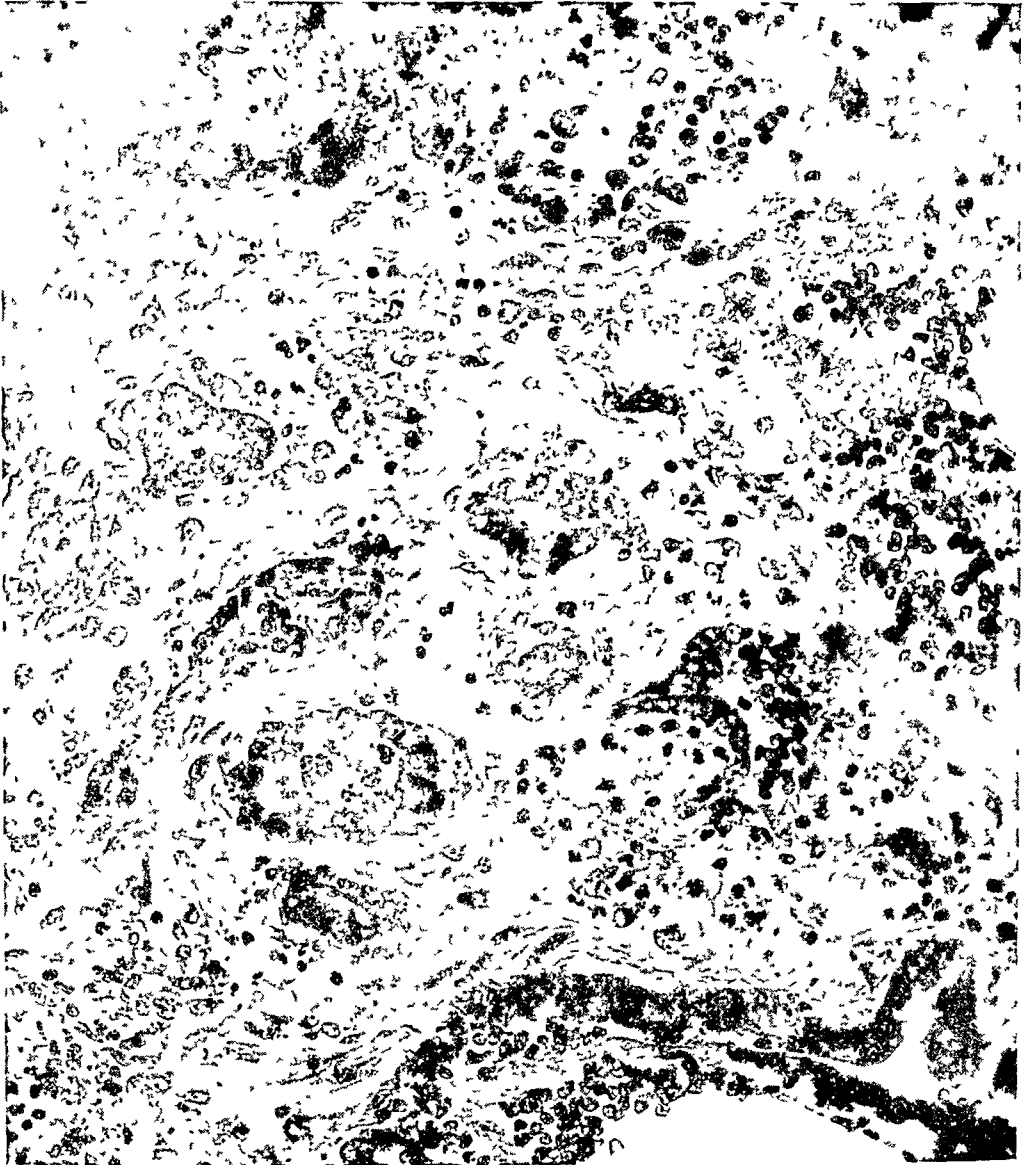


Fig. 17.—The cuboidal cells lining the glandular structures were interpreted by older pathologists as alveolar epithelium that had "reclaimed" its fetal cuboidal aspect. In reality these are cells of bronchiolar origin. The same cells, single and the groups, can be seen scattered throughout the photomicrograph. (Patient studied at the Beth Israel Hospital, Boston). Hematoxylin and eosin;  $\times 300$ .

spicuous in normal tissues, "reclaim" their ancestral features in pathologic conditions, taking a fetal cuboidal aspect. The so-called "alveolar

cell" cancers of the lung were traced to these structures.<sup>43</sup> In reality the "glandular structures" observed in sclerosed lungs are nothing other than "alveoli" lined by cells of bronchiolar origin (fig. 17). This condition observed in the sclerosed lung is a counterpart of what one sees in atrophic (Laënnec's) cirrhosis of the liver, in which there also occurs a new formation of bile ducts in the connective tissue encircling the hepatic lobules.

As mentioned, the view of the mesenchymal nature of the lining of the air sacs is at present shared by many pathologists. But even those who still maintain that the alveoli are lined by epithelial cells have come to accept my conception of the "defensive and metabolic" functions of the alveolar cells, which is indeed the essential. It is interesting that these writers regard the histiocytic potentialities of the "respiratory epithelium" as merely an exception in the bodily economy. In this respect, the opinion of Renan<sup>44</sup> may be quoted. In his inauguration discourse at the Academie Française, where he was elected to take the chair of Claude Bernard, Renan said: "*Le mot d'exception est anti-scientifique. Ce qu'on appelle exception est un phénomène dont une ou plusieurs conditions sont inconnues.*" ("The word 'exception' is anti-scientific; what is called an exception is a phenomenon, one or more conditions of which are not known.")

#### SUMMARY

Infection of rabbits with the bovine type of tubercle bacilli by way of the trachea provokes an instantaneous reaction in the lungs.

The early lesion, that is, the "primitive tubercle," is found in the lung within five minutes after the aerogenous infection. The lesion is intra-alveolar, being a "parenchymatous alveolitis."

The cells that primarily respond to the acid-fast bacilli are those commonly designated as "respiratory epithelium." Under the influence of Koch's bacillus, these cells undergo instantaneous morphologic changes, proliferate, separate from the wall of the air sacs, phagocytose the acid-fast bacillus and form typical miliary tubercles. They also form giant cells of the Langhans type.

Rabbits infected with bovine tubercle bacilli by way of the trachea survive from three to four times longer than those infected with the same amount of bacilli by way of the blood stream. In many of the aerogenously infected animals the acid-fast bacilli remain apparently

43. Fried, B. M.: Primary Carcinoma of the Lungs; Bronchiogenic Cancer, Baltimore, Williams & Wilkins Company, to be published.

44. Renan, Ernst: *L'Œuvre de Claude Bernard*, Paris, Baillière, 1881.

“immured for life” in the lungs, for no lesions or bacilli are detected in organs outside the pulmonary tissues.

The pulmonary reaction to the “virtual” infection with the Koch’s bacillus is akin to that of the “model infections” with vital dyes and oils reported previously.<sup>1</sup>

# THE SITES OF DECALCIFICATION AND OF BONE LESIONS IN EXPERIMENTAL HYPERPARATHYROIDISM \*

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Recent clinical and chemical studies have demonstrated the ease with which the mineral constituents of the bony skeleton are mobilized and depleted in a variety of conditions. Few discussions exist concerning the varying degrees of susceptibility to decalcification of the various portions of the bony skeleton or of individual bones. Nor are the relations between decalcification in various sites and the corresponding pathologic pictures much discussed. With these relations in mind, we examined histologically the carpal, tarsal, metacarpal, metatarsal, phalangeal and other bones (like the apophyses and sesamoids) of guinea-pigs with hyperparathyroidism, in addition to the bones previously investigated, such as the long tubular bones, ribs, skull and jaw. The distribution of lesions in the bones of young guinea-pigs suffering from experimental scurvy was also studied and compared with that in guinea-pigs showing hyperparathyroidism.

## THE DIFFERENTIAL GROWTH OF BONE

Certain features in the embryogenesis and postnatal development of the skeleton have a distinct bearing on the interpretation of our pathologic observations and on the conclusions based on them. To some degree, the succession of events in the development of the skeleton of man and that in the ordinary laboratory animal run parallel. Strong<sup>1</sup> drew certain analogies between the order of ossification in the rat and that in man. It is well known that certain bones reach their final forms and complete maturity much sooner than others. While species differ-

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\* From the Laboratory Division, Hospital for Joint Diseases.

\* Aided by a grant from the Herbert L. Celler Research Fund.

\* The parathormone used in the experiments was supplied by Eli Lilly and Company.

1. Strong, R. M.: *Am. J. Anat.* **36**:313, 1925.

ences exist in this respect, the progress of ossification within a given species is constant.

Ossification begins in man in the clavicle when the embryo is 15 mm. long (crown-rump) and 39 days of age. Numerous centers of ossification for the formation of other bones appear rapidly thereafter, and at birth the diaphyses of all the tubular bones are formed. At the time of birth, there are no centers of ossification in the cartilaginous epiphyses of the long tubular bones, except in the lower end of each femur, where a center is generally present, and in the upper end of each tibia, where one is occasionally present.<sup>2</sup> The epiphyses are formed by endochondral ossification. This progresses after the centers of ossification appear; most of the cartilaginous mass is soon replaced by bone. Further growth of the epiphysis is slow after this, and takes place along the line of junction of the bone and the remaining cartilaginous portion of the epiphysis. Finally, when the bony epiphysis is completely formed, an irregular cement line marks the junction between the bone and the articular cartilage, along which there is slow progressive erosion of the cartilage and formation of new bone by creeping replacement. The spongy trabeculae of the epiphysis are composed of fine-fibered lamellar bone, and no haversian canals are present except in the larger ones. The reconstruction of these fully formed trabeculae also proceeds slowly.

The epiphyses of the short tubular bones (metacarpals, metatarsals and phalanges) ossify rapidly, reaching their complete maturity not long after puberty; the epiphyses and diaphyses of these bones fuse and the epiphyseal cartilage plates disappear. On the other hand, fusion of the epiphyses of the lower ends of the femurs, of the upper ends of the tibiae and of the upper ends of the humeri does not occur in man until at least the nineteenth or the twentieth year. Like the epiphyses, the tarsal and carpal bones, with the exception of the os calcis, are entirely formed by endochondral ossification. These bones ossify rapidly after the appearance of centers of ossification in them, and thereafter grow slowly at the periphery beneath the cartilage. The exception, the os calcis, has an epiphysis at its posterior extremity.

On the metaphyseal surfaces of all epiphyseal cartilage plates new bone is formed as long as the plates remain active, but bone formation is most abundant in these regions in bones of greatest growth. There is a progressive diminution leading finally to complete cessation of ossification, as the epiphyseal cartilage plates go through the stages of closing and disappearing. In man, closure of the epiphyseal cartilage plate is followed by its rapid disappearance, resulting in fusion of the epiphysis and diaphysis, but in certain animals the plate may close without disap-

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2. Adair, F. L., and Scammon, R. E.: *Am. J. Obst. & Gynec.* **2**:35, 1921.

pearing for a long time. Dawson<sup>3</sup> showed that in the rat the epiphyseal cartilage plates of the upper ends of the humeri, of the lower ends of the femurs and of the upper ends of the tibiae remain until senility.

Bone formation proceeds very actively at the costochondral junctions until maturity. Furthermore, it may be noted that the jawbone is almost continually in a state of active growth and transformation. This is because it is subject so constantly to the mechanical influences of mastication. The skull continues to grow along the lines of suture until these close. The suture lines in the skull correspond to some degree to the epiphyseal plates of the tubular bones.<sup>4</sup>

This summary of the embryogenesis and postnatal development of the skeleton shows that bone formation and growth proceed at different rates in the various bones, and even in various portions of the same bone. In certain sites very active formation of bone occurs for a long period, as, for instance, in the metaphyses of the long tubular bones, the costochondral junctions, the jaw and the vicinity of the sutures of the skull bones. The junctions of cartilage and bone in the epiphyses, especially of the long tubular bones, are sites of somewhat slower but active formation of bone. When skeletal maturity is reached the growth of the bone ceases, and there is a slowing down of the rate of formation of bone at the sites at which it was previously most active. However, a number of these sites of early active formation and transformation of bone remain capable of again resuming these processes, even during adult life. In regard to the shafts of long tubular bones, it is of interest to observe that in early postnatal life the periosteum is of very great importance in the formation of bone, but as the animal becomes older there is considerable diminution in the functional capacities of the periosteum, which becomes dormant.

#### EXPERIMENTAL RESULTS

Decalcification is the only established specific effect of parathyroid extract (parathormone) on bone, and the resorption is generally evidenced by the presence of enlarged haversian canals or by lacunae containing osteoclasts on the periosteal and endosteal surfaces of bone. It is therefore possible to follow by histologic methods the complete anatomic distribution of the changes induced by such treatment. A young guinea-pig, weighing 300 Gm., into which 20 units of parathyroid extract per hundred grams of body weight has been injected, will show in forty-eight hours the greatest amount of resorption at the sites of most active growth of bone, that is, in the metaphyses of the long tubular bones, at the costochondral junctions, in the shaft cortices (most intense at the

3. Dawson, A. B.: *Anat. Rec.* **31**:1, 1925.

4. Mair, R.: *Ztschr. f. d. ges. Anat. (Abt. 1)* **90**:293, 1929.



encoche or terminal portion of the cortex and diminishing as the cortex of the middle of the shaft is approached). The jaw shows intense decalcification and resorption.

In the study of the bones of a large number of animals suffering from experimental hyperparathyroidism and showing consistently the histologic changes described, we were impressed by the equally consistent absence of these changes in spongy bone<sup>5</sup> of the epiphyses of the long tubular bones. We therefore undertook to determine whether changes could be induced in the epiphyses and in bones formed like the epiphyses in guinea-pigs weighing 300 Gm. by the injection of much larger amounts of parathormone.

Three guinea-pigs of this weight were given single doses of 50, 100 and 165 units of parathyroid extract per hundred grams of body weight. Extensive resorption, with the presence of osteoclasts and Howship's lacunae, was found at the



Fig. 1.—The costochondral junction of a rib of a young guinea-pig weighing 300 Gm. The animal was killed forty-eight hours after the injection of 500 units of parathyroid extract. Figures 1 to 6 all represent effects produced in this animal. Here it is to be noted that the cortex is fractured and the bone disintegrated, that the columns of endochondral bone are splintered, that new endochondral ossification has ceased, and that there is considerable hemorrhage in the marrow;  $\times 40$ .

5. Roux (Die Entwicklungsmechanik, Vortr. u. Aufs. ü. Entwicklungsmech. d. Organ., 1905, nos. 1 to 5) classified the components of spongy bone as follows: (1) tubes (tubuli), (2) hemispheric spaces (pilae), (3) plates (lamellae) and (4) beams (trabeculae). The tubuli ossei are the long spaces in spongy bone enclosed by the thicker trabeculae, and the pilae ossei are curved spaces in spongy bone, particularly underneath the articular cartilage, and are enclosed by the thinner lamellae. Any classification of the elements of spongy bone must be a rough one, because there are innumerable transition forms and shapes that make a strict terminology impossible (Jaffe, H. L.: Structure of Bone with Particular Reference to Its Fibrillar Nature and Relation of Function to Internal Architecture, Arch. Surg. 19:24, 1929). The term "trabeculae" is frequently used as a synonym for the term "spongy bone."

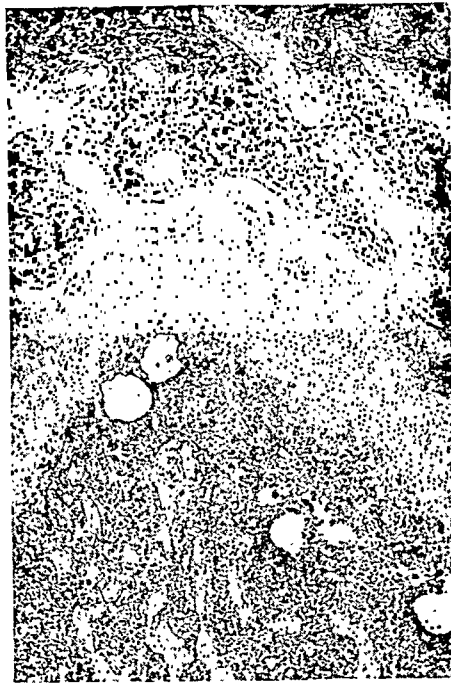


Fig. 2.—The lower end of the femur;  $\times 22$ . There are considerable resorption of bone and fibrosis of marrow in the metaphysis; a number of small cysts are present beneath the epiphyseal cartilage plate. No such changes are observed in the epiphysis above the plate, where the marrow is normal.

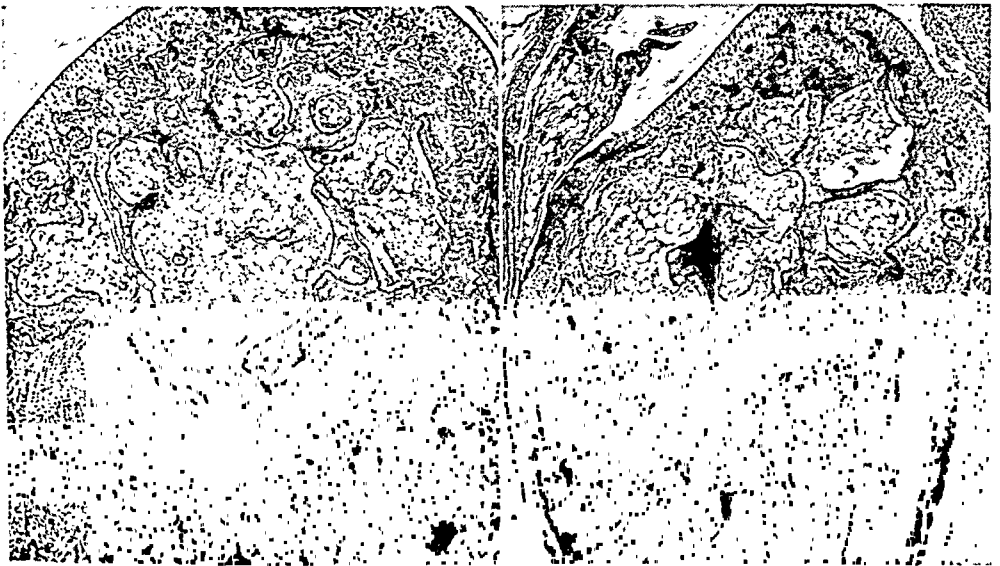


Fig. 3.—At the left are the epiphysis and part of the metaphysis of a metatarsal bone;  $\times 22$ . The marrow of the epiphysis is fatty and no abnormal resorption is observed. Some fibrosis of the marrow and osteoclastic resorption of the bony trabeculae in the metaphysis is seen, but the changes are less than in the corresponding region in figure 2.

At the right are the epiphysis and the metaphysis of a phalanx;  $\times 22$ . The epiphysis appears normal, but the metaphysis shows some osteoclastic resorption. The changes are less than in the metatarsal at the left.

costochondral junctions, in the metaphyses, in the shafts of the ribs and rapidly growing long tubular bones, and in the skull and jawbones. However, the spongy bone of the epiphyses and of the carpal and tarsal bones of these animals showed a striking absence of change. At most, it showed only simple thinning of the elements.

The shaft cortices showed an amount of resorption directly proportional to the growth capacity of the bones, the cortices of the ribs and long tubular bones showing much resorption, and those of the metacarpal, metatarsal and phalangeal bones little or none. In two of these young guinea-pigs, the endochondrally formed patella showed evidences of active resorption following the injection of 100 and 165 units of parathyroid extract per hundred grams of body weight. The reason

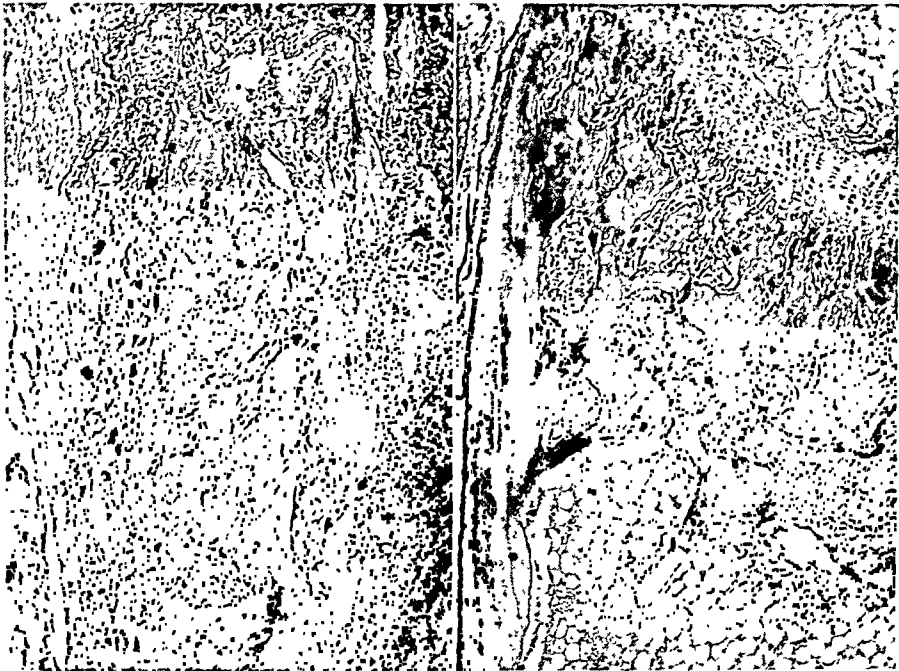


Fig. 4.—At the left is the encoche or terminal portion of the cortex of the shaft of the lower end of the femur;  $\times 85$ . Note the large number of osteoclasts and the connective tissue in the enlarged canals of the blood vessels. Resorption is very active, as this is an area of rapid growth. At the right is the encoche or terminal portion of the cortex of the shaft of a phalanx;  $\times 85$ . Note that resorption and fibrosis are absent here. Compare with the corresponding region in the rapidly growing bone at the left.

for this probably lies in the fact that the patella does not begin to ossify until relatively late and, because of the active progress of ossification, responds more like the spongy bone of the metaphyses than like that of the epiphyses and of the tarsal or carpal bones (figs. 1 to 6).

We also studied the distribution of lesions in the bones of young guinea-pigs receiving repeated, stepped-up doses of parathyroid extract over a period of twenty days.

Six guinea-pigs, weighing between 270 and 320 Gm., received injections of gradually increased doses of parathyroid extract, finally receiving 60 units daily for the last four days. The distribution of the lesions was the same as in the

young guinea-pigs receiving 1 large dose. No osteoclastic resorption or fibrous tissue formation was found in any of the epiphyses or in the carpal and tarsal bones. The shafts of the slowly growing short tubular bones showed less resorption when compared with the shafts of the rapidly growing long tubular bones, and the lesions in the metaphyses were less prominent than in the long tubular bones.

In line with the finding that the resorption effects of parathyroid extract are most marked in those portions of the skeleton that are most



Fig. 5.—Above is a section through the cortex of the middle of the shaft of the humerus;  $\times 85$ . Note the active subperiosteal resorption and the enlargement of the canals of the blood vessels. A Volkmann canal is observed penetrating the entire thickness of the cortex.

Below is the cortex of the shaft of a metacarpal bone;  $\times 85$ . The bone is compact and resorption is not observed. Compare with the cortex of the rapidly growing bone above.

actively growing is the observation that the bones of adult guinea-pigs are relatively much less susceptible to the effects of a single dose of parathyroid extract than those of young animals.<sup>6</sup> However, when very

6. Jaffe, H. L.; Bodansky, A., and Blair, J. E.: Fibrous Osteodystrophy (Osteitis Fibrosa) in Experimental Hyperparathyroidism of Guinea-Pigs, Arch. Path. **11**:207, 1931; J. Exper. Med. to be published.

great doses are administered to adult guinea-pigs, resorption may be found where formation of bone still occurs, though this is reduced to a state of slight activity. Thus, if the encoche is still present, resorption and fibrosis will be found in it. Since maturity of the guinea-pig is accompanied by a diminution in the number of the haversian canals in the shaft cortices, resorption, when it occurs in the cortices, appears beneath the endosteum and periosteum, but mostly beneath the endosteum. It is actually to the greatest degree beneath the endosteum, which in adult life has greater osteogenic capacity in the normal transformation of bone.



Fig. 6.—Part of a section of a tarsal bone;  $\times 85$ . Note the absence of marrow changes and the absence of resorption of the spongy bone. This bone responds to parathyroid extract like an epiphysis.

Seven adult guinea-pigs received daily injections of parathyroid extract for from seven to eleven days. They received a total of from 200 to 540 units of parathyroid extract during this period. All died from acute hyperparathyroidism. The histologic examination showed that with such treatment changes in bone and marrow could be produced in adults; the degree varied directly with the total dosage of parathyroid extract given, but the adults that were younger showed relatively more changes in bone and marrow, on a given dosage, than those that were older. The lesions, wherever present, although not severe, showed the greatest degree of advancement in the same sites as in the animals receiving large single doses. It should be observed, however, that none of the metacarpal, metatarsal or phalangeal bones showed any subperiosteal or subendosteal resorption. The carpal and tarsal bones, with the exception of the os calcis, showed complete absence of the resorption effects of hyperparathyroidism. The epiphyseal ends of the long

tubular bones showed practically no changes in the marrow or spongy bone. The youngest of these adults showed some osteoclasts and Howship's lacunae on the bone near the articular cartilages.

In three animals, in which care was taken not to increase the dose too rapidly, and to intermit the treatment when they appeared sick from hyperparathyroidism, injections of parathyroid extract were continued for sixty-nine days, with gradually increased dosage. Two of these guinea-pigs received a total of 3,040 units during this period; the third animal received more rapidly increasing doses during the last thirteen days of the treatment, and was given a total of 4,020 units. Bone

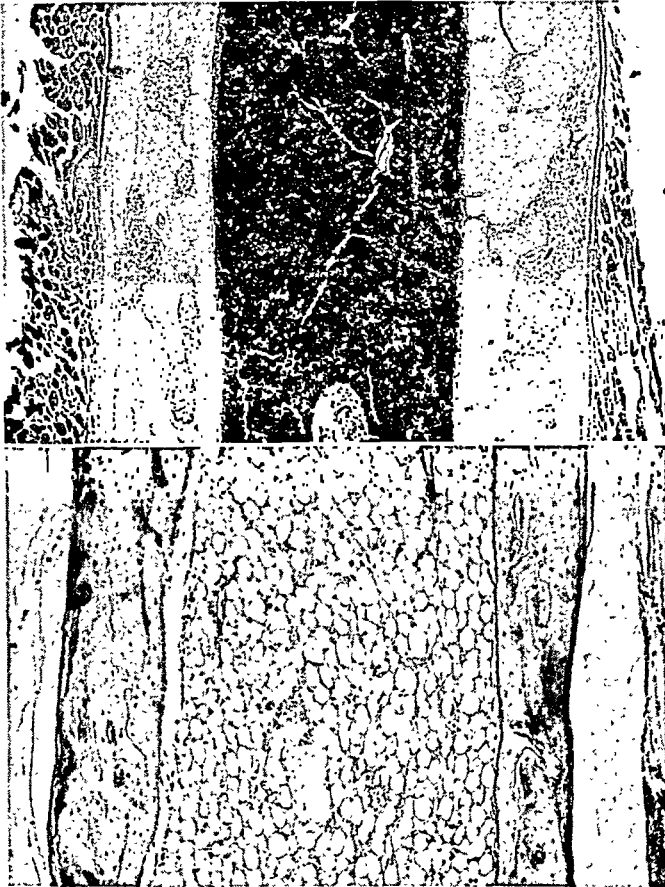


Fig. 7.—Above is the cortex of the middle of the shaft of the femur of an adult guinea-pig that received increasing doses of parathyroid extract for sixty-nine days. The animal received 3,040 units. The cortex shows marked enlargement of the canals of the blood vessels, which are filled with connective tissue and show osteoclasts in Howship's lacunae;  $\times 40$ .

Below is a section through the cortex of the middle of the shaft of a metacarpal bone from the same animal;  $\times 40$ . No lesions are observed in the cortex of this slow-growing bone. Compare with the femoral cortex above.

changes were produced, characterized by extensive enlargement of the haversian canals, which contained connective tissue and Howship's lacunae with osteoclasts. In these animals, however, resorptive changes appeared in the spongy bone of the epiphyseal ends of some of the long tubular bones and of the short tubular bones,

and in the cortices of these bones, especially those of the long tubular bones. Thus, these adult guinea-pigs were the first that showed any extensive involvement of the epiphyses of either the long or the short tubular bones. The carpal and tarsal bones showed slight evidences of resorption—insignificant compared with the epiphyses (fig. 7).

#### COMMENT

The experiments described showed that the resorption of bone in hyperparathyroidism occurs in certain sites of predilection. The increased susceptibility of these sites may be related to their rate of bone formation. It may be stated as a general principle that the sites of most active formation of bone are the sites most susceptible to physiologic or pathologic resorption of bone. Thus, those portions of the skeleton most susceptible to decalcification are those in which formation of bone is most active.

As we pointed out in the summary of the embryogenesis and post-natal development of bone, the tarsus, carpus and formed epiphyses of a young guinea-pig are sites of relatively slow growth, and are therefore least susceptible to the effects of hyperparathyroidism. These bones therefore respond to parathyroid extract in a manner similar to that of the bones of the adult skeleton. As the animal becomes older, and full growth in the length of the bones is completed, the bones or portions of bones that are most susceptible to resorption in the young animal become less so. When the animal is fully adult, the entire skeleton, although less susceptible, shows more or less uniformity in the response to the decalcifying effects of parathyroid extract.

On the other hand, Bauer, Aub and Albright<sup>7</sup> stated that in resorption of bone the calcium of the trabeculae<sup>8</sup> is "labile" and is drawn on in the first instance, while the calcium of the compact bone ("the structural parts of the bone") becomes available only "in the case of unusual body demands." They arrived at their conclusions partly through a study of the effect of parathyroid extract (parathormone) on rabbits, kittens and rats. After continued administration of parathyroid extract to three 5 week old rabbits for ninety-one days, during the major part of which (from fifty-six to seventy-four days) the rabbits received 8 units daily, the trabeculae were reported as diminished in number, but no effect was noted on the cortex of the bone either by gross or by roentgen examination. In two kittens 26 days of age, the administration of an average of 25.4 units of parathyroid extract daily for fifty-six days did not result in any greater reduction of the trabeculae than was observed in the course of the growth of bone in the normal controls. Four rats, 10 days of age at the beginning of the experiment, received

7. Bauer, W.; Aub, J. C., and Albright, F.: *J. Exper. Med.* **49**:145, 1929.

8. Bauer, Aub and Albright use the term "trabeculae" as a synonym for "spongy bone."

885 units each during a period of one hundred and ten days. Roentgen examination disclosed a diminution in length of the bones, but an increase in the number of trabeculae.

In spite of the fact that their data, as given here, do not seem to support their view, Bauer, Aub and Albright concluded that bone trabeculae are easily depleted by prolonged administration of parathyroid extract, and that these trabeculae serve as the source of readily available calcium. To a great degree their conclusions regarding the effects of parathyroid extract seem to have been inferred from the observation that adult cats show a depletion of trabeculae in the metaphyseal regions of the lower end of the femur and the upper end of the humerus following a diet low in calcium, and that following a diet high in calcium there is a reaccumulation of prominent trabeculae in these regions. Furthermore, they based their conclusions in part on the fact that intravital staining with alizarin red during the periods of feeding large amounts of calcium resulted in the deposition of the dye in the newly formed trabeculae. It is also interesting to note that no gross changes in the cortices of the shafts were reported by these authors, although some of their animals were kept on a diet low in calcium for a long period of time.

Regarding the effect of parathyroid extract on the bones of rabbits, we believe that Bauer, Aub and Albright should have taken into consideration the fact that the rabbit is a poor animal in which to follow changes in the spongy bone of the ends of the long tubular bones. Normally, relatively little spongy bone is present in these locations. In addition, a histologic study of the bones of animals is necessary before drawing conclusions regarding the absence of changes in the compact cortex. We have frequently found extensive resorption of cortical bone in our experimental animals on histologic examination, even when the gross examination revealed nothing striking. That this also holds true when roentgen examination of animal bones is employed as the sole means of judging decalcification is obvious. Furthermore, one may question the validity of generalizations concerning the physiologic functions of spongy bone in calcium metabolism, if not based on a histologic study of bones that contain considerable spongy elements, such as the patellae, vertebrae, tarsal and carpal bones, and the epiphyseal ends of the tubular bones.

On the basis of our experimental work, we believe that the widely accepted conception of Bauer, Aub and Albright is not tenable, and that the following formulation of the mechanism that determines the availability of calcium during resorption of the bones is preferable: Bone resorption and bone deposition are processes that go on in all bone constantly. When the rate of either of these processes is increased, it is increased in all bone. Both processes are more rapid in the regions of active growth, irrespective of anatomic structure. A mechanism is



therefore indicated for rapid decalcification in those regions when a condition is present favoring an excess of resorption over deposition. In regions of less active bone growth, resorption and decalcification proceed more slowly under normal conditions, and a great stimulus is required to produce a considerable degree of resorption. Thus, if we are to speak of labile calcium, it is the calcium in the regions of most active bone growth; if we are to speak of less readily available calcium, it is the calcium in the regions of less active growth.

This conception makes intelligible the pronounced susceptibility to resorption of the spongy bone of the metaphyses, especially those of the rapidly growing long tubular bones; of the costochondral junction; of the cortices of the shafts, particularly near the epiphyseal cartilage plates; of the cortices of the ribs used most in the respiratory act, and of the bones of the skull and of the lower jaw. It also explains why the metaphyses of the slower growing short tubular bones show relatively little evidence of resorption, while the cortices of the shafts of these bones show practically no evidence of resorption. At the same time, the spongy bone of all epiphyses and of the tarsal and carpal bones, when subjected to conditions producing the lesions described, show at the most only general thinning. Furthermore, this conception not only makes intelligible the greater susceptibility of the skeletons of actively growing young animals to parathyroid extract, compared with the skeletons of adult guinea-pigs, but explains also the quantitative differences in the response of different bones, and of different portions of given bones in animals treated with parathyroid extract. This is observed strikingly in young animals, but also to a certain degree in older animals.

It is well known that a supply of available calcium increases the hypercalcemic effects of parathyroid extract. We have demonstrated this in dogs under conditions in which the effect of recently absorbed (alimentary) calcium was eliminated.<sup>9</sup> Therefore the greater availability of the bone calcium in young animals is strongly indicated by the greater ease with which hypercalcemia is produced in young animals even of species (like the guinea-pig) which are relatively resistant to the effects of parathyroid extract.<sup>10</sup>

The conception of the quantitative relation of bone resorption to the rate of growth in particular sites may be further extended to include endosteal and periosteal resorption. In young, rapidly growing animals, with the shafts of tubular bones increasing in diameter, the marked degree of subperiosteal resorption that is observed in experimental

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9. Bodansky, A., and Jaffe, H. L.: *J. Exper. Med.* **53**:591, 1931.

10. Bodansky, A.; Blair, J. E., and Jaffe, H. L.: *J. Biol. Chem.* **88**:629, 1930.  
Bodansky, A., and Jaffe, H. L.: *J. Biol. Chem.* **93**:543, 1931.

hyperparathyroidism corresponds to the rapid growth in the subperiosteal regions in the normal animal. In the older animal, in which normally subperiosteal growth is greatly diminished, subperiosteal resorption is correspondingly less marked in hyperparathyroidism, while subendosteal resorption becomes by far the more prominent factor.

While active decalcification with the appearance of osteoclasts is probably the specific effect of parathyroid extract on the bones, this is marked only in the more rapidly growing portions of the bones. In the less rapidly growing portions, as for instance the middle portion of the diaphysis of a long tubular bone, a more generalized phenomenon of thinning is observed. We believe that this thinning underlies all resorption, in hyperparathyroidism as well as in osteoporosis, and affects the compact as well as the spongy bone. It is an expression of the tendency to spread an inadequate quantity of calcium over the entire mass of bone. This tendency is related to the growth stimulus, which is seen especially in the remarkable longitudinal growth of bone in spite of a calcium deficiency. This calcium deficiency may be of dietary origin or may be produced by such agents as parathyroid extract. The picture thus produced is analogous to the "smooth bone resorption," which was recognized by the earlier pathologists.<sup>11</sup>

In harmony with our view is also the old observation of Schmorl,<sup>12</sup> who in 1909 stated, in a discussion of clinical rickets, that the lesions appear earliest and are most severe in the regions of rapid growth, and the observation of many workers that experimental rickets is most easily produced in the rapidly growing animal.

Regions of rapid growth in bone, in agreement with our view, are also most susceptible to the effects of elementary phosphorus<sup>13</sup> and lead,<sup>14</sup> which cause the formation of thickets of spongy bone in these regions. The work of Park, Jackson and Kajdi<sup>14</sup> showed that lead, when taken for a sufficiently long period in sufficient dosage, can produce changes in bone, which are most marked where growth is occurring most rapidly, namely, at the anterior ends of the middle six ribs, at the lower ends of the femurs, at the upper ends of the humeri, at the lower ends of the radii and ulnae, and at both ends of the fibulae and tibiae. In two children, the bone changes produced by lead were well marked in the

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11. Jaffe, H. L.: Resorption of Bone; Consideration of Underlying Processes Particularly in Pathologic Processes, *Arch. Surg.* **20**:355, 1930.

12. Schmorl, G.: *Verhandl. d. Gesellsch. f. Nat. u. Heilk.*, 1908-1909, vol. 90.

13. Wegner, G.: *Virchows Arch. f. path. Anat.* **61**:44, 1847; **55**:11, 1872.  
Phemister, D. B.: Effect of Phosphorus on Growing Normal and Diseased Bones, *J. A. M. A.* **70**:1737, 1918.

14. Park, E. A.; Jackson, D., and Kajdi, L.: Shadows Produced by Lead in X-Ray Pictures of Growing Skeleton, *Am. J. Dis. Child.* **41**:485, 1931.

roentgenograms of the fast-growing parts of the skeleton, but were not evident in those of the slow-growing parts. The authors concluded that while "growth in bone occurs in many places, only where growth occurs rapidly do the changes readily reach such magnitude as to show in X-ray photographs."

It is suggested that the same principle applies in experimental scurvy, which shows the so-called characteristic lesions in the regions of active growth; the rest of the skeleton of young guinea-pigs on a scurvy-producing diet generally shows the nonspecific effect of the deficiency in the form of various degrees of thinning (simple atrophy). We reviewed the bones of young guinea-pigs suffering from experimental scurvy,<sup>15</sup> and found that the specific lesions were generally confined to the metaphyses, to the cortices of the shafts of the long tubular bones, and to the costochondral junctions. The spongy bone of the epiphyses, at the most, showed simple atrophy. However, if a 200 Gm. guinea-pig was placed on a scurvy-producing regimen, and if severe scurvy developed rapidly, lesions were also occasionally noted in the epiphyses.

It is interesting that a number of clinical diseases of bone occur in the epiphyses or in bones that are formed like the epiphyses. These are the so-called osteochondritides—Perthes' disease, Osgood-Schlatter's disease, Kienböck's disease, Köhler's disease, etc. It is probable that their long clinical course and their slow tendency to heal are related to the localization of lesions in bones (and in regions of bones) of slow metabolic exchange and therefore of slow reparative capacity.

#### CONCLUSIONS

From a study of the bones of guinea-pigs with experimental hyperparathyroidism we conclude that the rate of decalcification of the bony skeleton at any site is related to the rate of bone growth and metabolic exchange at that site. Decalcification and reactive lesions are more prominent in experimental hyperparathyroidism in regions of normally active growth of bone and less prominent in regions of less active growth. In the latter, simple thinning of the bone is sometimes the only change observed.

This conception makes intelligible the greater susceptibility of young animals to parathyroid extract and the reported details of the changes in the different bones and in the various portions of certain bones in experimental hyperparathyroidism.

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15. Jaffe, H. L.: *J. Infect. Dis.* **40**:502, 1927.

# EFFECTS OF INTRATHECAL ADMINISTRATION OF MERCUROCHROME-220 SOLUBLE AND OF METAPHEN \*

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Intrathecal injections of dyes and other chemicals have long been used in anatomic and physiologic studies. Quincke,<sup>1</sup> for instance, injected mercuric sulphide intrathecally, Reiner and Schnitzler<sup>2</sup> potassium ferrocyanide, Hill<sup>3</sup> methylene blue and Lewandowsky<sup>4</sup> sodium ferrocyanide, in studying the circulation of the cerebrospinal fluid. It is only recently, however, that the intrathecal injection of chemicals has been tried as a therapeutic measure in such conditions as syphilis, epilepsy and meningitis.

A variety of antiseptics have been studied both for their efficacy in sterilizing the meninges and for their toxicity. Seager<sup>5</sup> used compound solution of cresol in human subjects in 1902, and Wolff<sup>6</sup> in 1915 tried strong silver protein. Flexner and Amoss<sup>7</sup> in 1916 used both compound solution of cresol and strong silver protein in monkeys and found them to be harmful. They concluded that not much can be expected from the chemical treatment of epidemic meningitis and therefore pinned their faith on an immunologic attack; i. e., specific serums.

Fleteau and Handelsman,<sup>8</sup> in 1916, employed methenamine, Chinese india ink, a colloidal suspension of silver, silver nitrate, formaldehyde and iodine, intrathecally, all of which they found to be harmful.

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1. Quincke, H.: *Arch. f. Anat. u. Physiol.*, 1872, p. 153.

2. Reiner, M., and Schnitzler, J.: *Abstr., Centralbl. f. Physiol.* **8**:684, 1894.

3. Hill, L.: *Physiology and Pathology of the Cerebral Circulation*, London, 1896.

4. Lewandowsky, M.: *Ztschr. f. klin. Med.* **40**:480, 1900.

5. Seager: Quoted by Flexner and Amoss (footnote 7).

6. Wolff, G.: *Deutsche med. Wchnschr.* **41**:1486, 1915.

7. Flexner, S., and Amoss, H. L.: *J. Exper. Med.* **23**:683, 1916.

8. Fleteau, E., and Handelsman, J.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **31**:1, 1916.

Wegeforth and Essick,<sup>9</sup> in 1919, working on cats with experimentally produced meningitis, employed acriflavine, potassium permanganate, potassium biniodide, phenol, silver nitrate, benzoyl alcohol, mercuric iodide and compound solution of cresol. Continuous lavage, intermittent lavage and simple injection of the drug were tried. None of these drugs was devoid of toxic effects, even when used in low concentrations.

Kolmer,<sup>10</sup> in 1926, reported using ethylhydrocupreine hydrochloride, mercurochrome-220 soluble, metaphen, gentian violet, acriflavine and rivanol in the treatment of pneumococcus meningitis in dogs, with disappointing results. Stewart,<sup>11</sup> in 1927, used ethylhydrocupreine along with serums and lavage in the treatment of pneumococcus meningitis in dogs, with encouraging results. Moldenshardt,<sup>12</sup> in 1929, reported the use of colloidal silver in purulent meningitis. Many other antiseptics have been tried, and all of them have been found to cause a meningeal reaction. This fact, per se, however, constitutes no objection to the use of chemicals intrathecally, since even serums cause a similar reaction, the so-called "serum meningitis." In fact, Goldman,<sup>13</sup> in 1930, found that even the common preservative used in serums, cresol U. S. P., caused a definite though not a severe pleocytosis when injected intrathecally in its commonly employed concentration (0.4 per cent).

Of the current chemical antiseptics, mercurochrome-220 soluble and metaphen have received the most attention in recent literature as non-toxic effective germicides. We therefore deemed it advisable to investigate the toxicity of these drugs injected intrathecally, with a view toward employing them later in the treatment of meningitis. Our problem was (1) to determine their intrathecal sublethal and subtoxic dosages, (2) to study the morbid anatomy following such injections, and (3) to determine whether intrathecally tolerated doses of these chemicals were bactericidal or efficacious in sterilizing the subarachnoid space.

#### TECHNICAL CONSIDERATIONS

The work reported here was all done on dogs. Because of the smaller size of the subjects and because of certain anatomic differences, subthecal punctures in dogs are generally more difficult to do and more

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9. Wegeforth, P., and Essick, C. R.: *J. Pharmacol. & Exper. Therap.* **13**:335, 1919.

10. Kolmer, J. A.: *Pneumococcus and Streptococcus Meningitis*, *J. A. M. A.* **92**:874, 1929; *Chemotherapy and Serumtherapy of Pneumococcus and Streptococcus Meningitis*; *Résumé of Present Status of Treatment of Septic Meningitis*, with *Recommendation of Method*, *Arch. Otolaryng.* **3**:481, 1926.

11. Stewart, F. W.: *Local Specific Treatment of Experimental Pneumococcic Meningitis*, *J. A. M. A.* **89**:1316, 1927.

12. Moldenshardt, H.: *München. med. Wchnschr.* **76**:1875, 1929.

13. Goldman, D.: *Serum Meningitis*, *Arch. Path.* **9**:1027, 1930.

fraught with danger than those in man. Ochsner, Gage and Cutting<sup>14</sup> stated that it is virtually impossible to perform successful lumbar puncture on the dog. We have not found this to be the case, but we must admit that the operation calls for a great degree of skill, is somewhat uncertain, and is frequently attended by injury to the animal. Knowledge of the anatomy involved is therefore essential before attempting sub-theal punctures on this animal.

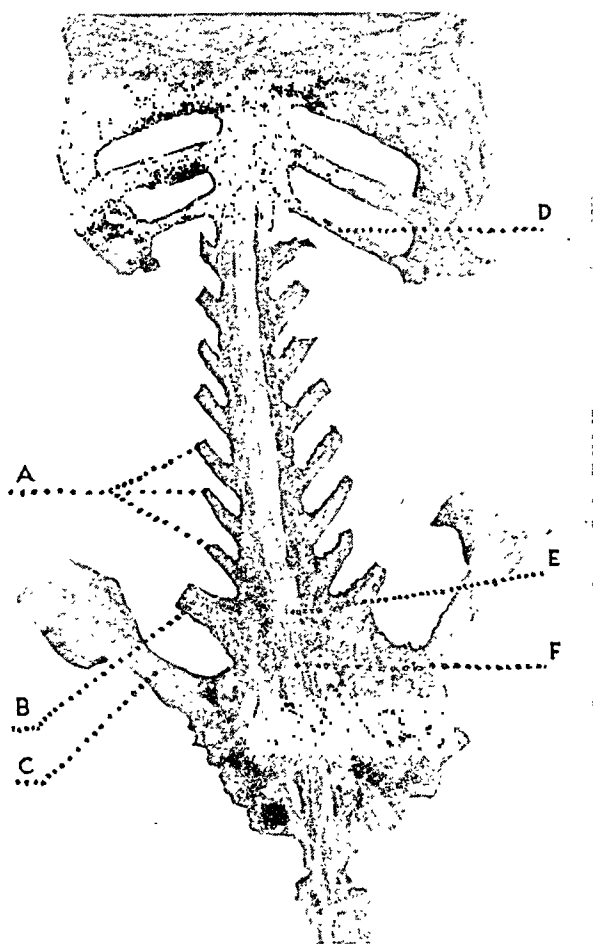


Fig. 1.—The anatomic relations of the lumbar cord: *A*, fifth, sixth and seventh lumbar processes; *B*, iliac crest; *C*, femur; *D*, last rib; *E*, end of cord, and *F*, cauda equina. The top of the vertebral column has been removed, exposing the cord, which extends to the level of the seventh lumbar vertebra. Note the relation of the iliac crests to this level.

In the dog there are, as a rule, seven lumbar vertebrae (fig. 1) and occasionally only six, as compared to five in man. The spinous processes

14. Ochsner, A.; Gage, I. M., and Cutting, R. A.: Comparative Value of Splanchnic and Spinal Analgesia in Treatment of Experimental Ileus, *Arch. Surg.* 20:802, 1930.

are directed slightly cephalad instead of caudad as in man. The cord extends down to the level of the body of the last lumbar vertebra, instead of to the second lumbar vertebra as in man, narrowing rapidly into the conus at the level of the sixth or the seventh lumbar vertebra. The cauda equina extends down to the lower level of the sacrum and is much shorter than in man.

An imaginary line drawn through the iliac crests intersects the lumbar column at the sixth interspace. Lumbar punctures can be performed through this space, the space above it or the space below it; i. e., through the fifth or the sixth lumbar, or through the lumbosacral interspace. Punctures above this level are inadvisable, because of the danger of injuring the cord.

The dura in the lower lumbar region is more loosely adherent to the bony wall (because of the relatively thicker pad of epidural fatty tissue) than in the upper lumbar and thoracic regions, and yields easily before the incoming needle, with the result that a dural snap may not be felt when the needle enters the canal. For this reason, also, dry taps are not infrequent, as the dura may "give" and not be pierced by the needle. A similar condition in the human new-born infant and prematurely born infant has been described by Glaser<sup>15</sup> to explain the difficulty in obtaining fluid by lumbar puncture in these infants.

The low extent of the cord accounts for the frequency of bloody taps and also for the occasional occurrence of a transverse myelitis following simple lumbar puncture.

To keep the animal quiet and thus to minimize the danger of injuring the cord an anesthetic should be used. We employed morphine-ether anesthesia.

For lumbar puncture, a short bevel needle about 7 cm. in length and 22 gage, fitted with an obturator, was used. The anesthetized animal was placed on its left side, the back flexed, and the needle inserted into the sixth lumbar interspace just to one side of the midline and directed forward, cephalad and medially, at an angle of about 45 degrees with the frontal plane, 30 degrees with the sagittal plane, and 45 degrees with the transverse plane, being guided by the body of the spinous process till the posterior arch was reached. As has been pointed out by Dvorak and Manson,<sup>16</sup> the lumbar vertebrae in the dog are too closely approximated to allow a passage of the needle between them in the midline, except in the lumbosacral space. Laterally, however, the interspinous space is amply wide. Through this lateral aperture, gentle probing being used, if necessary, the needle reaches the subdural space usually about from 3 to 5 cm. from its point of entrance into the skin. The cerebrospinal fluid usually trickles slowly out of the needle; occasionally it will gush out, and at other times it will slowly drip out.

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15. Glaser, J.: Cerebrospinal Fluid of Premature Infants, with Special Reference to Intracranial Hemorrhage and Pigmentation, *Am. J. Dis. Child.* **36**:195, 1928.

16. Dvorak, H., and Manson, M. H.: *Proc. Soc. Exper. Biol. & Med.* **28**:344, 1930.

For cisternal puncture, a needle about 5 cm. in length and about 22 gage, fitted with an obturator, was used. In doing cisternal punctures, we prefer the lateral position of the dog, the same as for lumbar puncture, to the prone position used by Goldman.<sup>13</sup> The occipital protuberance was palpated with the index finger and the protuberance of the axis with the thumb of the left hand, and the needle was inserted midway between these points, being directed upward and forward at an angle of about 45 degrees, and directly into the cistern.

The skull from the occipital protuberance to the border of the foramen magnum, in the dog, slants more obliquely forward than in the human being. This should be kept in mind if the indirect method of cisternal puncture—that is, feeling one's way along the skull with the needle—is used. The cistern in the dog is relatively smaller than that in the human being, so that injury to the medulla is much more likely to occur. The cervical cord extends for a distance of about from 0.5 to 1 cm. into the cranial cavity, so that if the needle is not directed obliquely forward and upward, injury to the cervical cord may ensue. In spite of all precautions, however, bloody fluids are not uncommon following lumbar or cisternal puncture.

In addition, we have observed the following clinical symptoms and sequelae attending simple lumbar puncture:

1. Jerking of the feet or tail when the cord or nerves were touched by the needle.
2. Listlessness, anorexia, asthenia, ataxia and hyperesthesia of the lower half of the body for twenty-four hours after the puncture.
3. Transverse myelitis due to injury of the cord.
4. Subarachnoid block, causing subsequent dry taps, arachnoid adhesions being found post mortem.
5. Local subcutaneous hematoma at the site of puncture.

Subsequent to cisternal puncture, the following sequelae have been noted:

1. Death due to puncture of vital centers in the medulla.
2. Respiratory embarrassment, i. e., dyspnea, apnea and Cheyne-Stokes' respiration, due to hemorrhage about the cistern.
3. Asthenia, ataxia, tremors, irritability, loss of equilibrium and anorexia for twenty-four hours after the puncture.

Since the introduction intrathecally of very small amounts of material can enormously raise the intracranial pressure, the following precautions were observed when such injections were made:

The solution was injected at a temperature of about 40 C., and at a pressure of from 20 to 25 cm. water. Pressure readings before and during the injection were taken by means of a manometer that was attached either to a two-way stop-cock connected to the puncture needle or directly to a second needle placed into the subarachnoid space for this purpose.



When cistern-lumbar lavage was performed, the temperature of the solution was controlled by means of a simple apparatus described by one of us.<sup>17</sup> The pressure was controlled by raising or lowering the reservoir containing the solution, since the fluid was allowed to flow in by gravity. In order to avoid injury to the medulla due to movement of the cisternal needle, we used a combination guard and retainer on the cisternal needle, whenever cistern-lumbar lavage was done.

*Necropsy.*—The removal of the cord and brain intact in a dog is a tedious, but frequently a necessary, procedure. After experimenting with several methods of performing this part of the necropsy, such as sawing off the top of the vertebral column and skull, using a double saw rachitome to divide the transverse processes, using trephine openings in removing the skull and several other variations, we evolved the following technic, which we believe to be the simplest, quickest and most efficient. By this method it is possible for one person to remove the brain and cord intact in from fifteen to twenty minutes.

The dog's body is placed prone on a flat table. The skin is split the whole length of the back from the snout to the tail and then the subcutaneous tissues and muscles are incised along the whole length of the vertebral column, the knife passing along each side of the spinal column in the groove between the spinous processes and the uppermost projections of the transverse processes. The temporal muscles are dissected away from the skull. The transverse processes on each side of the spine are then divided by means of a single blade rachitome. The blade is placed in the groove on either side of the spinous processes and is sharply struck with a heavy wooden mallet. Then, with the use of a sequester or bone forceps, the top of the vertebral column can easily be lifted off by starting at the sacrum and ripping upward to the skull. The remaining edges of the bone can be trimmed, if necessary, with a bone-cutter. The skull is then split by three blows, one with the rachitome blade placed transversely just posterior to the frontal sinuses and the other two with the blade placed successively on each side of the skull, in the temporal fossae parallel to the sagittal suture and about midway between the midline and the external auditory meatus. The skull can then be lifted off, leaving only a centimeter or so of thin bone over the medulla, which can easily be removed with a bone-cutter. The spinal cord with its dura and the brain are then removed intact in retrograde fashion by beginning with the cauda and dissecting upward. The cord and brain may then be fixed in formaldehyde pending further examination.

#### MERCUROCHROME

*Toxicity.*—We attempted to determine the maximum tolerated doses of mercurochrome-22 soluble, H. W. & D. (disodium-monohydroxy-mercuri-dibromfluorescein) both by intraspinal and intracisternal injection in dogs. At the start we were unaware of the great toxicity of this drug when injected intrathecally. It soon became apparent that the usual 2 per cent or even the 1 per cent mercurochrome was too toxic. Therefore, we resorted to the use of 0.25, 0.10 and 0.025 per cent solu-

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17. Perlstein, M. A.: J. Lab. & Clin. Med. **16**:837, 1931.

tions (1:400, 1:1,000 and 1:4,000). The solution was injected slowly by means of a syringe, being continuously mixed with cerebrospinal fluid by a process of sufflation during the course of the injection.

Table 1 indicates the great toxicity of mercurochrome when injected into the cistern of a dog. Of seven dogs, in all of which there was no postmortem evidence of hemorrhage or trauma due to the puncture itself, only two failed to manifest any toxic symptoms. These two dogs received the smallest doses, 0.055 and 0.066 mg. per kilogram, respec-

TABLE 1.—*Summary of Experiments with Intracisternal Injection of Mercurochrome in Dogs*

Dog*	Weight of Dog, Kg.	Amount and Concentration of Solution Injected	Amount Mercurochrome Injected, Mg.	Amount Mercurochrome per Kg., Mg.	Results	Structural Changes
1	4.5	1 cc. of 0.025% (1:4,000)	0.25	0.055	No toxic symptoms; complete recovery; dog had mange and died 1 wk. later	No stain left; hyperemia of cord and brain
2	25.0	1.65 cc. of 0.10% (1:1,000)	1.65	0.066	No toxic symptoms; complete recovery; dog killed 13 days later	No stain left; dilated central canal; otherwise normal
3	10.9	1.2 cc. of 0.10% (1:1,000)	1.2	0.110	Listlessness for 24 hrs.; complete recovery; dog killed on seventh day	No stain left; organizing serous leptomeningitis
4	17.2	1 cc. of 0.25% (1:400)	2.5	0.145	Dog died respiratory death within 35 minutes	Stain covers all of cord and base of brain; very little over cortex and none in lateral ventricles; otherwise grossly normal
5	6.5	1 cc. of 0.10% (1:1,000)	1.0	0.154	Toxic symptoms (Cheyne-Stokes' respirations, convulsions, etc.) lasting 1 hr.; complete recovery	(Dog ran away six weeks later)
6	16.2	1 cc. of 1% (1:100)	10.0	0.616	Dog died respiratory death with convulsions within 12 min.	Stain covers cord and base of brain, but not cortex or ventricles; moderate hyperemia
7	14.1	2.5 cc. of 2% (1:50)	50.0	3.542	Dog died respiratory death within 5 min.	Stain as in dog 6; hyperemia

\* One dog is omitted because postmortem evidence of trauma to the medulla was found.

tively. One that received 0.110 mg. per kilogram was listless for twenty-four hours, but made a complete recovery. Another that had received 0.154 mg. per kilogram underwent a severe reaction, hovering on the lethal, but finally recovered without residual ill effects. In the other animals that received 0.145 mg. per kilogram or more, death ensued within from five to thirty-five minutes, depending on the dosage employed.

The minimum lethal dose of mercurochrome, when injected into the cistern of the dog, would therefore be between 0.110 and 0.145 mg. per kilogram, while the maximum subtoxic dose would be between 0.066 and 0.110 mg. per kilogram.

When injected into the lumbar spine (table 2), the drug was less fatal than when injected into the cistern. In fact, a dose as high as 1.21 mg. per kilogram, the highest dose used, failed to kill the animal. Nevertheless, it was very toxic, the subtoxic dose being 0.17 mg. per kilogram. Of five dogs receiving 0.22 mg. per kilogram or more of mercurochrome intraspinally, all manifested reactive symptoms, such as

TABLE 2.—*Summary of Experiments with Intraspinal Injection of Mercurochrome in Dogs*

Dog	Weight of Dog, Kg.	Amount and Concentration of Solution Injected	Amount of Mercurochrome, Mg.	Amount of Mercurochrome, per Kg.	Results	Structural Changes
1	14.3	1 cc. of 0.10% (1:1,000)	1.0	0.07	No toxic symptoms; complete recovery; dog killed 11 wk. later	Small subdural hemorrhage in lumbar cord; otherwise normal
2	8.8	1.5 cc. of 0.10% (1:1,000)	1.5	0.17	No toxic symptoms; complete recovery; dog killed 1 mo. later	Hyperemia of cord and brain; organizing serous meningitis
3	7.3	1.6 cc. of 0.10% (1:1,000)	1.6	0.22	Transverse myelitis developed, which improved so that on 11th day dog had only slight limp; killed on 11th day	Organizing serofibrinous meningitis of cord in lower thoracic to sacral region
4	10.4	1 cc. of 0.25% (1:400)	2.5	0.24	Immediate mild general reaction; transverse myelitis which did not improve in 6 wk., at which time dog was killed	Lumbar myelomalacia
5	9.1	2 cc. of 0.20% (1:500)	4.0	0.44	Transverse myelitis, which cleared up at end of 1 wk.; dog killed on 11th day	Focal myelomalacia of lumbar cord; organizing edema of lumbar subarachnoid space
6	10.0	1.1 cc. of 1% (1:100)	11.0	1.10	No transverse myelitis; dog gave birth to litter of pups on 7th day; was very weak and irritable, and lost weight; killed on 11th day	Acute fibrinopurulent meningitis
7	16.5	2 cc. of 1% (1:100)	20.0	1.21	Immediate convulsions and dyspnea; attack lasted 40 min.; transient palsy of upper extremity; transverse myelitis of lumbar cord, which did not improve; dog killed 1 mo. later	Lumbar myelomalacia

anorexia, ataxia, irritability and tremors. In addition, four of these showed clinical symptoms of transverse myelitis. We are convinced that the transverse myelitis in all of these animals was not the result of faulty technic, but due rather to the action of the drug proper, since there were no postmortem evidences of mechanical trauma, and since the incidence of transverse myelitis following simple lumbar puncture or following the injection of substances other than mercurochrome was much less, in our experience, than following the injection of mercurochrome. The maximum subtoxic dose of mercurochrome intraspinally, as evidenced in our work, is therefore between 0.17 and 0.22 mg. per kilogram.

The relatively greater toxicity of mercurochrome when injected intrathecally than when given intravenously had been previously pointed out by Kolmer<sup>18</sup> and by McKinley and Holden.<sup>19</sup> Kolmer determined the maximum single tolerated dose of mercurochrome for dogs by intravenous injection to be 35 mg., corresponding to 3.5 cc. of a 1 per cent solution per kilogram. When given intrathecally, he found the maximum single tolerated dose to be between 1 and 3 mg. per kilogram. In other words, he found that mercurochrome was from 12 to 35 times more toxic when injected intraspinally than when injected intravenously. These figures were all for normal dogs. In dogs suffering from meningitis, the intrathecal tolerance for mercurochrome, according to Kolmer, was even less, the maximum tolerated dose for repeated injections being between 0.1 and 0.3 mg. per kilogram, or about one tenth of that of a normal dog.

The figures given by Kolmer for the maximum tolerated dose of mercurochrome in normal dogs are about 10 times as high as those found by us. There is a possibility that the mercurochrome we used contained some impurity that might account for its greater toxicity. This is not surprising in view of the results of the chemical analyses of four commercial samples of mercurochrome by Eyre, Notton and Pope.<sup>20</sup> These observers found that whereas the percentage of mercury in the dry material should theoretically be 26.7 per cent, it was as high as 40.9 per cent in one sample and 11.4 per cent in another. Macht and Harden<sup>21</sup> found that the disodium salt of *dihydroxymercuri-dibromfluorescein* that was found as an impurity in the earlier batches of mercurochrome manufactured was much more toxic (by intravenous injection) than mercurochrome (disodium salt of *monohydroxymercuri-dibromfluorescein*) itself. Burn and Elphick<sup>22</sup> tested eight commercial samples of mercurochrome for their toxicity to mice by intravenous injection, and found that the most toxic sample was over 3.5 times as toxic as the least toxic. It is to be expected that such differences in toxicity would be even greater when the drug is injected intrathecally.

We did not attempt to check this point, for we were primarily interested in the action of the mercurochrome that was used in our hospital

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18. Kolmer (footnote 10, second reference).

19. McKinley, E. B., and Holden, M.: Dangers Attending Intraspinal Treatment with Mercurochrome, *J. A. M. A.* **88**:1391, 1927.

20. Eyre, J.; Notton, H. E. F., and Pope, W. J.: *Brit. M. J.* **3**:238, 1928.

21. Macht, D. I., and Harden, W. C.: *J. Pharmacol. & Exper. Therap.* **32**:321, 1928.

22. Burn, J. H., and Elphick, G. K.: *Quart. J. Pharm. & Pharmacol.* **3**:177, 1930.

service and in our laboratories, and because, in any event, it was quite evident that even the least toxic mercurochrome was too toxic a drug to be used intrathecally.

The greater toxicity of mercurochrome when injected intracisternally than when injected intraspinally is to be expected because of the proximity of the vital centers to the point of injection in the cisternal route. Kolmer's statement that the same dosages of mercurochrome are tolerated by intracisternal or intraventricular injection as by intraspinal injection has not been borne out by our work.

Our figures for the single maximum subtoxic doses in normal dogs are about the same as those given by Kolmer for repeated injections in dogs with meningitis. Since our work with mercurochrome to date has been done only on normal dogs, we have been unable to check his reported greater toxicity of the drug in the presence of meningitis. However, it is reasonable to expect that such a difference exists, because of the damaged condition of the meninges in meningitis. The lowered sugar content of the cerebrospinal fluid in purulent meningitis may also be a factor in the greater toxicity of the drug in the presence of a meningitis, in view of the observation by Macht and Harden<sup>21</sup> that by intravenous injection mercurochrome is less toxic when injected in combination with dextrose than when injected alone.

The only other reference we could find in the literature to the toxicity of mercurochrome for animals by intrathecal injection is by McKinley and Holden, who injected it intraspinally into rabbits. They found that doses as low as 0.4 mg. per kilogram, the lowest that they tried, caused convulsions and death. In the interpretation of these results in animals, it should be remembered, as has been pointed out by Young and his co-workers,<sup>23</sup> that by intravenous injection mercurochrome has the same toxicity for human beings as for rabbits, being slightly more toxic for dogs.

*Bactericidal Activity of Mercurochrome in Vitro.*—In order to determine whether sublethal doses of mercurochrome would be bactericidal, it was necessary to determine the bactericidal action of various dilutions of mercurochrome. Thalhimer<sup>24</sup> found that dilutions above 1:1,000 were not bactericidal to twenty-four hour broth cultures of *Bacterium typhosus* in fifteen minute exposures. Kolmer<sup>18</sup> found the range of final bactericidal dilution of mercurochrome in purulent cerebrospinal fluid containing pneumococci and streptococci to vary from 1:400 to

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23. Young, H. H.; Hill, J. H., and Scott, W. W.: Treatment of Infections and Infectious Diseases with Mercurochrome-220 Soluble; Analysis of 210 Cases that Furnish Many Definite Examples of Sterilisans Magna, Arch. Surg. **10**:813, 1925.

24. Thalhimer, W.: Personal communication to the authors.

1:1,000. Birkhaug<sup>25</sup> found the highest dilution of mercurochrome that would kill *Staphylococcus aureus*, *Streptococcus hemolyticus*, *B. coli*, *Gonococcus*, *B. anthracis* and *B. subtilis* to be 1:320. We found the highest dilution of mercurochrome in salt solution that would kill a twenty-four hour broth culture of *B. coli* after five minutes' exposure to be 1:1,000, using one loopful of a suspension of the organism to 0.5 cc. of the chemical. When dilutions were made with cerebrospinal fluid, instead of with salt solution, the results were the same, indicating that the cerebrospinal fluid itself has no significant bactericidal action. Since the maximum sublethal dose of mercurochrome by intracisternal injection is less than 0.145 mg. per kilogram, or for a 10 kilogram (22 pound) dog, 1.45 cc. of a 1:1,000 solution or for an adult person weighing 60 kilograms (132 pounds) about 8.7 cc. of a 1:1,000 solution or 0.87 cc. of a 1 per cent solution, the futility, as Kolmer has pointed out, of attaining by simple injection a bactericidal action in the subarachnoid space of any therapeutic value is evident.

*Lavage.*—Since by intraspinal and intracisternal injections such small quantities and concentrations of mercurochrome must be used as are barely bactericidal to begin with, and therefore certainly not bactericidal after dilution in the subarachnoid space, we decided to test out, by lavage from the cistern to the lumbar region, the toxicity of a 1:1,000 (0.1 per cent) solution of mercurochrome, that is, the highest dilution that we found to be bactericidal. It was thought that perhaps by this means of withdrawing part of the mercurochrome solution a sublethal bactericidal dose might be maintained in the subarachnoid space. Lavage from the cistern to the lumbar region was therefore performed in four dogs. In all there was a fatal outcome. The protocol of one of these experiments, which is illustrative of the others, follows:

Sept. 26, 1930: Dog P53P was used. The weight of the animal was 14.5 Kg. It was placed under morphine-ether anesthesia, and lumbar and cisternal punctures were performed. Clear fluids were obtained with cell counts of 3 lymphocytes and 1 lymphocyte, respectively (samples A and B, respectively).

At 3:37 p. m., treatment was started by lavage from a cisternal to a lumbar needle with 0.1 per cent mercurochrome in physiologic solution of sodium chloride at body temperature at a pressure of 22 cm. of water.

At 3:41 p. m., 4 cc. was collected at the lumbar needle, mercurochrome becoming visible in the lumbar fluid.

At 3:46 p. m., 9.5 cc. was collected (sample C taken).

At 3:50 p. m., 14.5 cc. was collected (sample D taken), fluid running in well.

At 3:53 p. m., 21 cc. was collected; the dog's respiration was a little deeper.

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25. Birkhaug, K. E.: Metaphen (4-Nitro-3, 5-Bisacetoxy-Mercuri-2-Cresol: Comparative Study of Commonly Used Disinfectants and Antiseptics; Histologic Changes Produced by Intravenous Administration of Metaphen in Rabbits, J. A. M. A. 95:917, 1930.

At 3:55 p. m., 28 cc. was collected (sample E taken), respiration becoming more rapid.

At 3:59 p. m., 36 cc. was collected (sample F taken). The breathing was irregular, gasping; the dog's head was kept retracted, and the body was stiff. The cisternal needle was removed twenty-two minutes after the start of the lavage and after 40 cc. of 0.1 per cent mercurochrome had been introduced into the cistern. Anesthesia was stopped.

At 4:01 p. m., 5.5 cc. more was collected from the lumbar needle after the cessation of the introduction of fluid, making a total of 41.5 cc. collected.

At 4:04 p. m., 1.5 cc. more was collected at the lumbar needle, making a total of 43 cc. collected; the fluid was coming very slowly, about 1 drop a minute; the needle was removed from the lumbar canal. The dog's respiration was a little better.

At 4:14 p. m., the animal was coming out of the ether. The head was still kept retracted; the respiration was still a little rapid, but regular.

Summary of Lavage: The elapsed time was twenty-two minutes; the amount introduced, 40 cc.; the amount removed, 36 cc. In the five minutes after stopping lavage, 7 cc. more was collected at the lumbar needle.

Colorimetry: With the use of a Duboscq colorimeter, the specimens taken at various stages during the lavage were compared with the original 0.1 per cent solution of mercurochrome for intensity of color: Sample C showed 26 per cent, sample D 36 per cent, sample E 59 per cent, and sample F 52 per cent, of the original color.

Bactericidal Activity: The last specimen (F) of lavaged fluid was tested for bactericidal action against a twenty-four hour broth culture of *B. coli*, the cerebrospinal fluid before lavage (sample A) and the 0.1 per cent mercurochrome solution being used as positive and negative controls, respectively. It was found that both the mercurochromized lavaged fluid and the original 0.1 per cent solution were bactericidal to these organisms after fifteen minute exposures. (It should be pointed out that a different strain of *B. coli* from that to which mercurochrome was found to be bactericidal only in 1:1,000 dilution was used in these tests.)

Sept. 27, 1930: The dog was unable to get up. It had been comatose since lavage. It moved its fore legs, but apparently not its hind legs. Ether anesthesia was given. Lumbar puncture yielded 2 drops of thick, blood-tinged fluid that did not flow through the needle. By cisternal puncture, 1.5 cc. of pinkish fluid was obtained that clotted immediately. Chemical examination of this fluid revealed the total nitrogen to be 0.49 Gm. per hundred cubic centimeters. The total protein was 3.06 Gm. per hundred cubic centimeters. Practically all of the protein consisted of albumin, there being only a minute quantity of globulin present.

Twenty hours after the lavage, the dog was moribund. It was then anesthetized and killed.

Postmortem Examination: A mercurochrome stain was present all over the cord and the base of the brain, but not over the hemisphere nor in the lateral ventricles. There was a moderate hyperemia of the blood vessels over the surface of the cord and brain. There were hemorrhages, varying in size from that of a pinpoint to that of a pinhead and in some places to that of a split pea, scattered throughout the white matter and involving practically all of it, and extending to a smaller degree into the gray matter of the cord, medulla and pons (fig. 2). The changes were greatest in the region of the medulla and cervical cord, gradually becoming less marked above and below this point, so that in the lumbar region there were only one or two such areas of hemorrhage, while the sacral and caudal

areas looked normal. There was a blood clot in the fourth ventricle extending up into the third, but not into the lateral ventricles. In one area it encroached on the cerebellar substance. There were no changes above the level of the optic chiasma, except for the presence of perivascular white streaks in the sulci at the base of the brain (exudate). Histologically, in addition to the areas of hemorrhage in the meninges and in the brain and cord, there was an acute purulent leptomeningitis.

The dilution of mercurochrome in the subarachnoid space is probably in the nature of a defensive mechanism, i. e., an attempt on the part of the choroid plexus and meninges to dilute a circulating poison by an increased output of cerebrospinal fluid. Colorimetric comparison of suc-

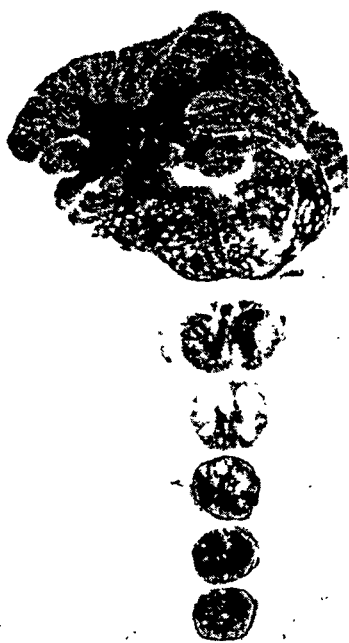


Fig. 2.—Sections of the brain and of the cord of a dog lavaged with 0.1 per cent mercurochrome in physiologic solution of sodium chloride. The sections of the formaldehyde-hardened cord and brain are, beginning at the top, through the fourth ventricle, upper cervical, upper thoracic, lower thoracic, lumbar and lumbosacral cord. The diffuse and punctate hemorrhages involve mainly the white matter, being most marked in the cervical region and becoming less marked caudad and cephalad. The actual size is shown.

cessive specimens of lavage fluid collected at the needle of exit with the original solution introduced, indicates that the intrathecal concentration of mercurochrome increases as the lavage progresses. This may possibly be interpreted as a gradual failure, through exhaustion, of the choroid plexus and meninges to produce sufficient cerebrospinal fluid to dilute the mercurochrome, with the result that a lethal concentration of the drug in the subarachnoid space is attained.



## METAPHEN

*Toxicity.*—There is no reference in the literature regarding the intrathecal toxicity of metaphen (4-nitro-3, 5 diacetoxy-mercuri-2-cresol). By intravenous injection, Raiziss and Severac<sup>26</sup> found that white rats tolerated the drug well in doses of 4 mg. per kilogram, and Birkhaug<sup>25</sup> reported that for rabbits doses of 3 mg. per kilogram, were well borne. Kolmer,<sup>27</sup> who used metaphen intrathecally in dogs, did not state how toxic it was by this route.

We used forty-one dogs in studying the effect of the intrathecal injection of metaphen. Twenty dogs were given the drug by the cisternal route, eleven by the spinal route and ten were lavaged with metaphen from the cisternal to the lumbar needle. In order to exclude as far as possible the factors of trauma and hemorrhage as causes of changes following the injection of metaphen, fifteen dogs in which punctures resulted in bloody fluids or in needle puncture trauma were omitted from the tables, and all brains and cords were examined for evidences of such trauma.

Table 3 is a summary of the results of the intracisternal injection of metaphen in fourteen dogs.

The maximum subtoxic dose was between 0.103 and 0.119 mg. per kilogram. The dogs receiving between 0.119 and 0.156 mg. per kilogram manifested toxic symptoms, but recovered, or apparently would have recovered if they had not been put to death. The one receiving 0.183 mg. per kilogram was killed when death impended thirteen days after the injection. One receiving 0.528 mg. per kilogram died on the third day. The minimum lethal dose of metaphen, when injected into the cistern, was therefore between 0.156 and 0.183 mg. per kilogram.

The animals that recovered manifested surprisingly few symptoms clinically to indicate that they had received a foreign substance intrathecally. Aside from a listlessness or inactivity that rarely amounted to stupor, anorexia and mild asthenia that lasted for one or two days, these dogs acted normally; no permanent after-effects were noted. In two dogs that received 0.095 and 0.119 mg. per kilogram, respectively, there occurred more severe reactions. They remained weak and ataxic up to the time that they were killed on the fourth day. In each of these dogs, however, cisternal hemorrhages were found post mortem that may have been of traumatic origin; but these dogs are included in the series because clear fluids were obtained at the time of puncture, and because it was doubtful whether the hemorrhages were the cause of the symptoms. Two dogs which had apparently fully recovered twenty-four hours after

26. Raiziss, G. W., and Severac, M.: J. Lab. & Clin. Med. 9:71, 1923; J. Infect. Dis. 40:447, 1927.

27. Kolmer (footnote 10, first reference).

TABLE 3.—*Summary of Experiments with Intracisternal Injection of Metaphen in Dogs*

Dog*	Weight of Dog, Kg.	Amount and Concentration of Solution Injected	Amount Metaphen Injected, Mg.	Amount Metaphen per Kg., Mg.	Results	Structural Changes
1	6.4	1 cc. of 1:10,000	0.1	0.016	No toxic symptoms; complete recovery; dog killed on 12th day	No changes
2	5.9	1 cc. of 1:10,000	0.1	0.017	No toxic symptoms; complete recovery; dog killed 16 days later	No changes
3	4.5	1.5 cc. of 1:10,000	0.15	0.033	During the injection dog stopped breathing, resuscitated; complete recovery; killed 1 mo. later	Fibrotic thickening of the meninges
4	7.3	1 cc. of 1:2,500	0.4	0.055	No toxic symptoms; complete recovery; dog killed 16 days later	Small epidural hemorrhage at level of cistern
5	6.8	0.5 cc. of 1:1,000	0.5	0.073	Weakness for 12 hr.; apparent recovery after 24 hr.; dog died during an attempted repuncture of cistern	Needle-track traumatic hemorrhage in medulla; subdural hemorrhage; serofibrinous leptomeningitis
6	7.8	1.5 cc. of 1:2,500	0.6	0.077	No toxic symptoms; complete recovery; dog killed on 3d day	Acute serofibrinous leptomeningitis
7	6.3	0.6 cc. of 1:1,000	0.6	0.095	Listlessness and surliness for 2 days; asthenia on 4th day; dog killed on 4th day	Traumatic hemorrhage in medulla and over base of brain; acute serofibrinous leptomeningitis
8	6.8	0.7 cc. of 1:1,000	0.7	0.103	No toxic symptoms; complete recovery; dog killed on 15th day	Edema and fibrotic thickening of the leptomeninges
9	12.7	1.4 cc. of 1:1,000	1.4	0.110	Dog walking about 24 hr. later; cistern repunctured and bloody fluid obtained; dog died the next day, apparently as result of second puncture	Subarachnoid hemorrhages over cord and base of brain; serofibrinous leptomeningitis
10	5.9	0.7 cc. of 1:1,000	0.7	0.119	Dog listless; able to walk on 2d day, but remained asthenic and ataxic, especially in hind legs; killed on 4th day	Fibrinopurulent leptomeningitis; small cisternal hemorrhage
11	7.7	1 cc. of 1:1,000	1.0	0.130	Dog apparently fully recovered when killed 24 hr. later	Fibrinopurulent leptomeningitis
12	6.4	1 cc. of 1:1,000	1.0	0.156	Dog apparently fully recovered when killed 24 hr. later	Acute serofibrinous leptomeningitis
13	5.5	1 cc. of 1:1,000	1.0	0.183	Dog stuporous for several days, finally lapsing into coma; killed when moribund on 13th day	Necrosis of small area in medulla; chronic healed fibrinous meningitis
14	7.6	4 cc. of 1:1,000	4.0	0.528	Dog lethargic; died on 3rd day	Acute serofibrinous meningitis

\* Six dogs, all of whom had histologic evidence of an aseptic leptomeningitis, were omitted from this series because death was definitely due to trauma at first puncture.

the injection, and which were repunctured at that time to obtain cerebrospinal fluid for examination, died. They were included in the series because necropsy revealed extensive basal and cisternal hemorrhages in both animals and, in one also a fresh needle-track hemorrhage in the medulla, which we attributed to trauma incurred at the second puncture.

When injected into the spine, metaphen was less lethal than when injected into the cistern. In fact, a dose of 0.220 mg. per kilogram

TABLE 4.—*Summary of Experiments with Intraspinal Injection of Metaphen in Dogs*

Dog	Weight of Dog, Kg.	Amount of Solution Injected	Amount Metaphen Injected, Mg.	Amount Metaphen per Kg. Mg.	Results	Structural Changes
1	6.6	1 cc. of 1:10,000	0.1	0.015	Complete recovery; no toxic symptoms; dog killed 8 wk. later	No changes
2	3.9	1 cc. of 1:10,000	0.1	0.026	Complete recovery; no toxic symptoms; dog killed 1 mo. later	No changes
3	6.8	2 cc. of 1:5,000	0.4	0.059	Complete recovery; dog killed 2 wk. later	Moderate hyperemia of cord, brain and leptomeninges
4	9.7	1.5 cc. of 1:2,500	0.6	0.062	Complete recovery; dog killed on 21st day	Early fibrinous leptomeningitis limited to cord region
5	6.8	1.5 cc. of 1:2,500	0.6	0.088	No toxic symptoms; complete recovery; dog died of pneumonia 9 days later	Edema of leptomeninges
6	8.2	2 cc. of 1:2,500	0.8	0.097	No toxic symptoms; dog apparently recovering; killed on 2d day	Edema of leptomeninges
7	8.7	3 cc. of 1:2,500	1.2	0.138	Complete recovery; no toxic symptoms; dog died 1 mo. later of pneumonia	Hyperemia of leptomeninges and cord
8	14.5	5 cc. of 1:2,500	2.0	0.138	Dog lethargic and died 10 hr. later	Fibrinopurulent leptomeningitis limited to cord region
9	6.8	1.5 cc. of 1:1,000	1.5	0.220	Dog listless for several days; complete recovery; killed on 21st day	No changes

\* Two dogs were omitted from the series because both had traumatic transverse myelitis.

(table 4) failed to kill the dog into which it was injected intraspinally, whereas one of 0.183 mg. per kilogram killed the animal that received it in the cistern. One dog that received only 0.138 mg. per kilogram died ten hours after the injection, whereas two other dogs that received 0.138 and 0.220 mg. per kilogram, respectively, did not die. We believe that this apparent contradiction was due to the fact that the first dog, which succumbed, was one of our earlier animals in which the injection was made without proper control of the pressure. In view of our later knowledge that even small injections may enormously increase the intracranial pressure, we believe that death in this dog was attributable not to the toxic effect of metaphen, but to increased intracranial pressure.

In only one dog were there clinical symptoms of a severe reaction. This was one which was given the maximum dose of 0.220 mg. per kilogram, and which was stuporous for several days afterward, but which finally completely recovered, being killed three weeks later.

The maximum subtoxic dose of metaphen by intraspinal injection was therefore between 0.138 and 0.220 mg. per kilogram.

*Lavage.*—Ten dogs were lavaged from the cistern to the lumbar region with metaphen. Both a 1:10,000 and a 1:25,000 dilution of metaphen in physiologic solution of sodium chloride were used.

The average time of a lavage was between twenty and thirty minutes, during which from 30 to 40 cc. of fluid was allowed to run into the cistern, and about from 50 to 80 per cent of this amount of fluid was collected at the lumbar region. It took between one and a half and three minutes for the fluid introduced at the cistern to appear at the lumbar region. This determination was made by adding some methylene blue to the original solution and noting the first appearance of the colored fluid at the needle of exit.

Lavage is attended with many accidents to the dog incident to the movement of the dog or to that of the needle during the course of the experiment, so that five of these dogs died either during the lavage experiment or within the following thirty-six hours as a result of medullary or cisternal trauma. Of the five that recovered, one died two weeks later of a confluent bronchopneumonia, and four were put to death six weeks later after their complete recovery. The symptoms manifested by the animals that recovered were generally the same as those following the simple injection of metaphen, i. e., listlessness and irritability, which generally disappeared in a few days. Although one dog that recovered was lavaged with a 1:10,000 solution, we are not certain that such a concentration is harmless when large amounts are employed. However, cistern-lumbar lavage with a 1:25,000 solution of metaphen was found to be well tolerated.

*Bactericidal Activity.*—Metaphen (4-nitro-3, 5-diacetoxymercuri-2 cresol) was introduced by Raiziss and Severac <sup>26</sup> as an antiseptic with a high bactericidal coefficient and a low toxicity. These observers found that in twenty-four hours it inhibited the growth of *S. aureus* in dilutions up to 1:20,400,000. In one minute exposures in a 50 per cent serum, metaphen destroyed this organism in a 1:10,000 dilution, being ten times more powerful than hexylresorcinol S. T. 37 and twenty-five times more powerful than mercurochrome under the same conditions.

Birkhaug,<sup>25</sup> comparing various germicides for their bactericidal activity against several organisms, obtained similar results. Compared with phenol as having a bactericidal coefficient of 1 for *B. coli* after forty-eight hours' incubation, he found that metaphen had a phenol coeffi-

cient of 250, hexylresorcinol one of 50, mercuric chloride one of 125, tincture of iodine one of 38 and mercurochrome one of 2. He found the highest dilution of metaphen in distilled water capable of killing *B. coli*, the most resistant of all organisms that he tried, after ten, but not after five, minutes' contact, to be 1:20,000, compared with 1:160 for mercurochrome.

When diluted in equal parts of blood serum and distilled water, all of the antiseptics showed markedly reduced antiseptic power. Under these conditions, Birkhaug found that metaphen was still a more effective bactericide than any of the other antiseptics that he used, being over sixty times more powerful than mercurochrome.

Thalhimer<sup>24</sup> found that for exposures of two and one-half minutes metaphen was bactericidal to a twenty-four hour broth culture of *B. typhosus* in dilutions up to 1:50,000. For fifteen minute exposures, he found it to be bactericidal in dilutions up to 1:100,000.

Since the presence of blood serum, according to Birkhaug, diminished the bactericidal power of metaphen to one tenth of its value when it was diluted in distilled water, we first determined whether cerebrospinal fluid had such an inhibitory effect.

We found that metaphen when diluted either in physiologic solution of sodium chloride or in equal parts of cerebrospinal fluid and physiologic solution of sodium chloride was bactericidal to twenty-four hour broth cultures of *B. coli* in five minute exposures in dilutions of 1:50,000 and in fifteen minute exposures in dilutions of 1:100,000. The average phenol coefficient of metaphen diluted in physiologic solution of sodium chloride was 642, the same as its phenol coefficient when it was diluted in 50 per cent cerebrospinal fluid. It is therefore apparent that normal cerebrospinal fluid has practically no effect on the bactericidal activity of metaphen. As compared with mercurochrome, which we found to be bactericidal in 1:1,000 dilution, under similar conditions, metaphen was found to be over 50 times more bactericidal.

#### CEREBROSPINAL FLUID CHANGES

The intrathecal injection of practically any substance is followed by changes in the cerebrospinal fluid. These changes were studied in twenty-eight dogs, twenty after the injection of metaphen and eight after the injection of mercurochrome.

Within one hour after the injection of the chemical, provided the dog did not die before this time, the cerebrospinal fluid manifested changes that persisted for from three to seven days, depending on the severity of the reaction. The fluid was tinged fluorescent pink for several hours after the injection of mercurochrome or yellow after the injection of metaphen. It was usually clear, but at times was turbid. The cerebrospinal fluid pressure was increased, except in those cases in which the

fluid was too thick and gelatinous to flow freely through the needle; in these cases, the pressure was decreased. Within twelve hours there occurred an increase in the protein content of the fluid, at times slight and at other times so great that the fluid coagulated almost immediately after withdrawal from the body. This increase in protein was practically all albumin, there being hardly any increase in globulin. The coagulum that formed was composed of fibrin, and contained many polymorphonuclear leukocytes, lymphocytes and erythrocytes.

The cell content of the fluid was increased. There was usually an exudation of erythrocytes first, as observed by Kolmer<sup>18</sup> following intrathecal injections of mercurochrome, up to several thousand per cubic millimeter. This initial exudation of erythrocytes probably accounts for the yellow color and positive benzidine reaction of the fluid obtained at subsequent punctures. The white blood cells were also increased in number and were both of the polymorphonuclear and lymphocytic type, the former predominating. There were frequently over 1,000 cells per cubic millimeter. In two cases, after injection of metaphen, many so-called endothelial cells were present also. In four instances in which metaphen was administered, and in which the cerebrospinal fluid contained an increase in protein so marked that the fluid coagulated within a few minutes after its withdrawal, the cell counts were relatively low, being 4, 13, 15 and 75, respectively. This phenomenon of albuminocytologic dissociation we have been unable to explain. It may be due to the same cryptogenic mechanism that is responsible for the similar changes occurring in the cerebrospinal fluid in the Froin syndrome of spinal sub-arachnoid block.

Cultures and smears made from the cerebrospinal fluid were repeatedly negative for organisms, indicating that the changes in the fluid were due to an aseptic meningitis. The cerebrospinal fluid changes enumerated occurred even when as little as 0.015 mg. of metaphen per kilogram (the lowest dose that we used) was injected intrathecally.

#### PATHOLOGY

The brains and cords were fixed in 4 per cent formaldehyde and histologic sections were stained with hematoxylin-eosin and sudan III, and by the method of Pal-Weigert. In addition, bacterial stains were made when indicated.

Following the injection of mercurochrome or of metaphen, as following the injection of practically any substance into the subarachnoid space, there occurred a meningeal reaction.

This reaction was observed to vary from simple hyperemia and edema of the meninges to serofibrinopurulent aseptic leptomeningitis, depending on the amount of the drug injected, the individual susceptibility of the animal to the drug and the length of time elapsed between the injection and the necropsy.

Grossly, the cords and brains showed scant changes, even when the clinical symptoms were severe. At times there was a small localized subdural hemorrhage at the site of puncture, and in the more severe meningeal reactions perivascular white streaks in the sulci were visible, especially over the base of the brain. Frequently there was hyperemia of the blood vessels of the brain and cord.

After the injection of mercurochrome, the distribution of the dye over the cord and the brain varied with the site of the injection and the



Fig 3—Edema of the spinal subarachnoid space eleven days after intraspinal injection of mercurochrome. There is practically no cellular reaction; hematoxylin and eosin;  $\times$  about 120.

amount injected. In general, it was found over the cord, the medulla and the base of the brain. When the injection was made into the cistern, the dye had a wider distribution over the base of the brain and the cortex than when injected into the spine. Sometimes, with larger amounts, the stain partially covered the cortex. Only when very large quantities were injected, however, were the lateral ventricles stained. The order of frequency of distribution of the dye when injected intrathecally was, cord, medulla and pons, base of brain, cortex and finally ventricles. The

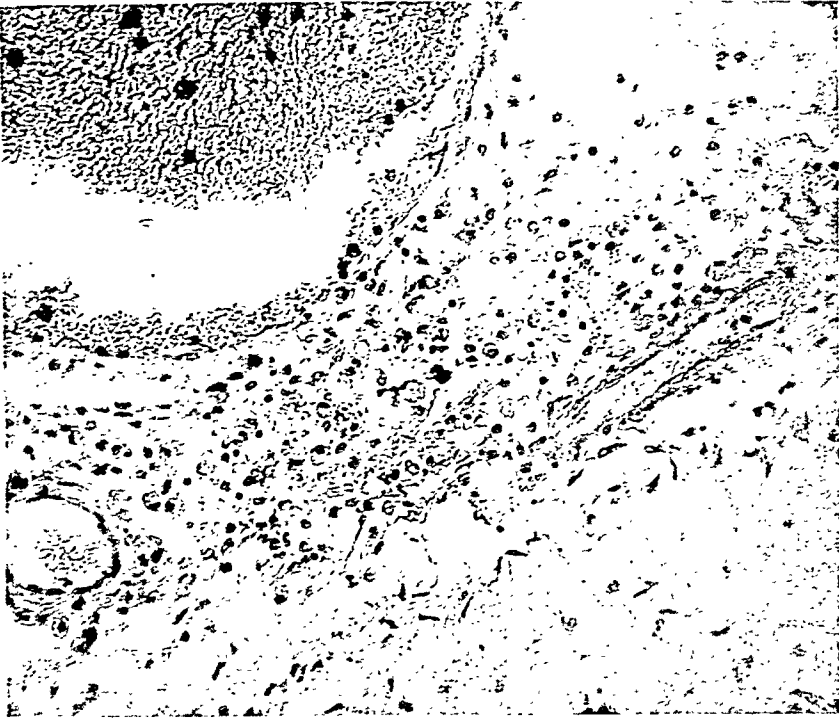


Fig. 4.—Acute aseptic serous meningitis five days after injection of mercurochrome. There are many endothelial and relatively few polymorphonuclear cells present in the subarachnoid space; hematoxylin and eosin;  $\times$  about 200.

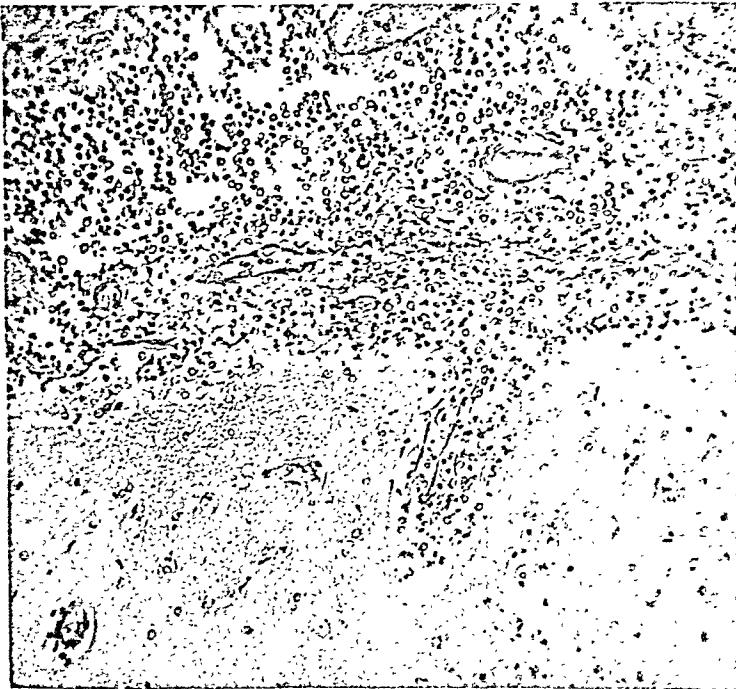


Fig. 5.—Purulent exudate in the leptomeninges twenty-four hours after injection of mercurochrome; hematoxylin and eosin;  $\times$  about 120.



stain did not appear to penetrate below the pia mater and disappeared within a few days.

Histologically, however, there were evidences of the presence of an aseptic meningeal reaction even in animals receiving minimal doses of the drugs, the cords and brains of which appeared grossly normal. The changes were essentially the same regardless of the drug used.

Early there occurred a hyperemia with some diapedesis of erythrocytes into the subarachnoid space. The spaces were distended by edema or by fibrinous exudate (fig. 3). There was usually an increased



Fig. 6—Organizing fibrinopurulent meningitis eleven days after intraspinal injection of mercurochrome. The predominating cells are fibroblasts, polymorphonuclear leukocytes and endothelial cells; hematoxylin and eosin;  $\times$  about 180.

number of polymorphonuclear leukocytes, lymphocytes and endothelial cells (fig. 4). In the more severe reactions there was a frankly purulent exudate in the subarachnoid space (fig. 5). If the dogs were killed from five to six days after the injection, there were usually many fibroblasts and plasma cells present in the leptomeninges (fig. 6). After several weeks, all that was left to indicate that a meningeal reaction had occurred was a fibrotic thickening of the meninges. Frequently, if the dog was killed from two to three weeks after the injection, no residual pathologic changes were discernible.

In those dogs in which transverse myelitis occurred, there were degeneration of the white matter as seen in Pal-Weigert stains and replacement of the gray matter by débris, granule cells and glial cells (figs. 7 and 8). In one dog in which clinical transverse myelitis developed, the symptoms were explained on the basis of a localized fibrinous

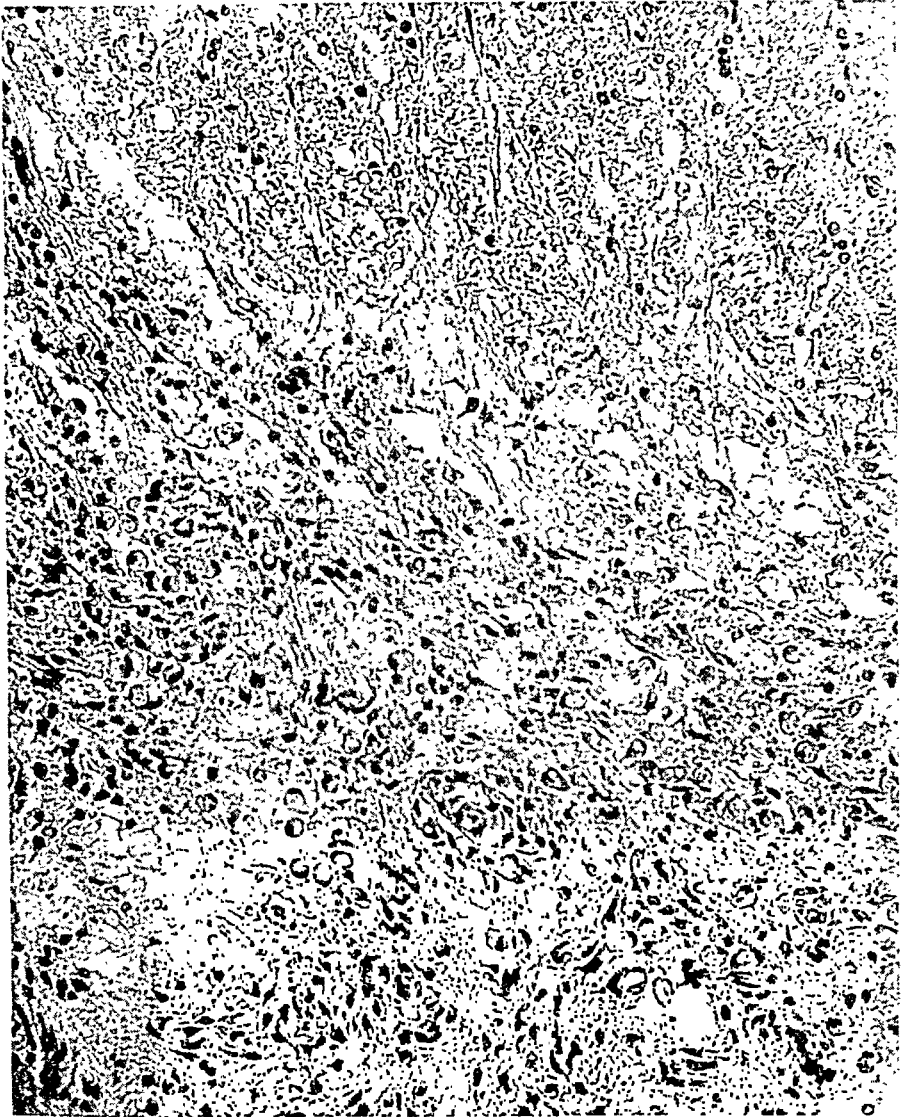


Fig. 7.—Necrosis of the gray matter of the cord eleven days after intraspinal injection of mercurochrome. The normal tissue is replaced by granule cells and glial cells; hematoxylin and eosin;  $\times$  about 200.

meningitis of the cord. Except for hyperemia, there were no other changes in the parenchyma of the cord and brain. Bacterial stains revealed no organisms. Except for an occasional small fat droplet in the meninges and about the central canal, sudan III-stained sections also showed no morbid changes.

In dogs that received lethal doses of mercurochrome into the cistern, except for the stained leptomeninges, the histologic conditions were entirely normal. This may have been because death occurred before detectable changes had time to occur.

As described, dogs in which lavage with a 0.1 per cent mercurochrome was performed frequently had, in addition to the meningeal reaction, changes in the cord and brain stem consisting of disseminated areas of hemorrhage and necrosis.

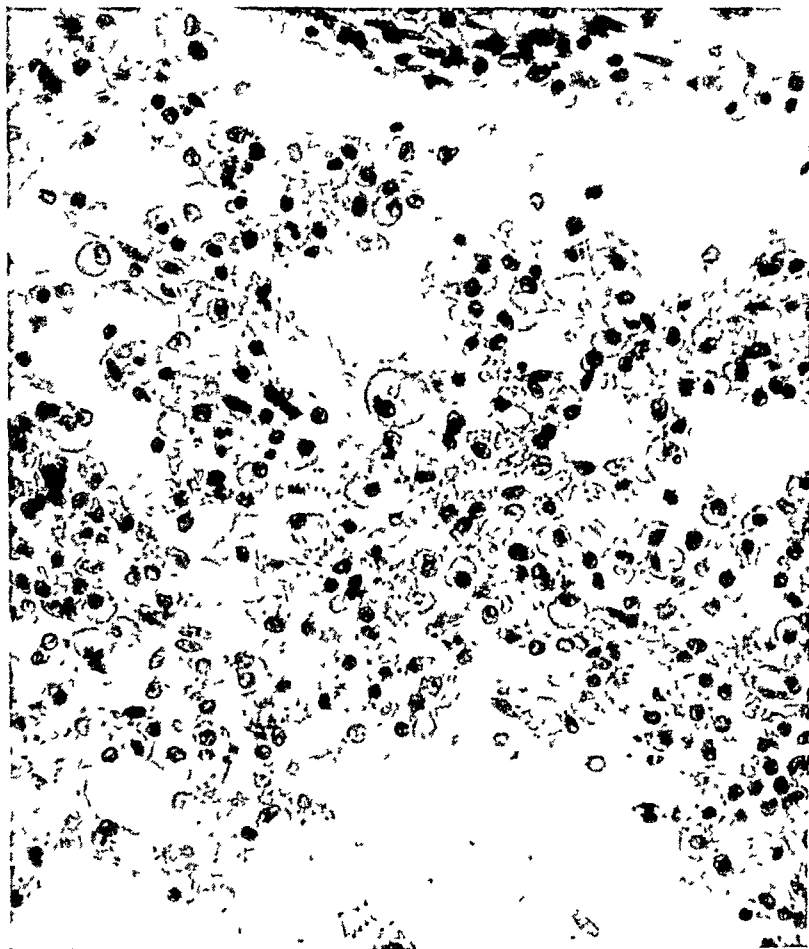


Fig 8.—Higher magnification of granule cells in necrotic foci. These are large, pale cells with a latticed cytoplasm and with an eccentrically placed oval nucleus; hematoxylin and eosin;  $\times$  about 350

#### COMMENT

In our search for a powerful antiseptic with a low intrathecal toxicity, we studied the effect of the intrathecal injection of mercurochrome and of metapen. It became apparent early in our work that the strength of the chemical was not so much a factor as the dosage in milligrams per kilogram of body weight. Thus, dogs that were treated by injection of 1:1,000 solution of metapen did not show any more severe symptoms

than those that were treated by injection of a 1:10,000 solution, provided the dose in milligrams per kilogram was approximately the same.

The dosages in milligrams per kilogram are given in table 5, which summarizes the results that we obtained with these chemicals. Equivalent dosages are also given for an adult human being weighing 60 kilograms (132 pounds). It is evident that both these drugs have a high intrathecal toxicity, both being somewhat less toxic when injected into the spinal subarachnoid space than when injected into the cistern. Of the two, metaphen is the less toxic and less lethal. Not only are its intracisternal subtoxic and sublethal doses less than those of mercurochrome, but the margin of safety between the subtoxic and the lethal dose is greater for metaphen. Moreover, we observed that the dogs receiving

TABLE 5.—*Comparison of Maximum Subtoxic and Minimum Lethal Doses of Mercurochrome and Metaphen*

Method	Maximum Subtoxic Dose		Minimal Lethal Dose	
	Mercurochrome	Metaphen	Mercurochrome	Metaphen
Intracisternal injection				
Mg. per Kg. body weight.....	0.07-0.11	0.10-0.12	0.11-0.15	0.16-0.18
Cc. of 1:1,000 solution for 60 Kg. (132 lb.) adult human being.....	4.0 -6.6	6.0 -7.2	6.6 -8.7	9.6 -10.8
Intraspinal injection				
Mg. per Kg. body weight.....	0.17-0.22	0.14-0.22	(Highest dose used, 1.21 Mg. per Kg., not fatal)	(Highest dose used, 0.22 Mg. per Kg., not fatal)
Cc. of 1:1,000 solution for 60 Kg. (132 lb.) adult human being.....	9.9 -13.2	8.3 -13.2		

lethal doses of metaphen intracisternally, usually died from one to thirteen days after the injection, apparently as a result of the severe aseptic meningitis. However, those that succumbed to the injection of mercurochrome into the cistern usually died a respiratory death, within thirty minutes after the injection, indicating that there was a direct action of the drug on the vital medullary centers. The fact that the medullary nuclei appeared normal on histologic examination does not disprove this theory.

Intraspinaly, practically the same subtoxic doses were found for both drugs. Minimum lethal doses by this route were not given, because the animals survived the highest intraspinal doses that we gave. However, mercurochrome is a more dangerous drug to use intraspinaly, for myelomalacia was much more common, following the intraspinal injection of mercurochrome than following that of metaphen.

In making up our dilutions of mercurochrome and metaphen for lavage, physiologic solution of sodium chloride was used. The question naturally arises as to what part the salt solution played in the production of the clinical and morbid changes, for it has been shown by Weed

and Wegeforth<sup>28</sup> that physiologic solution of sodium chloride itself is harmful when injected intrathecally. In view of the fact that none of a preliminary series of dogs in which we performed lavage with greater amounts of physiologic solution of sodium chloride showed nearly so severe reactions as those manifested by the dogs lavaged with either mercurochrome or metaphen, we feel convinced that these chemicals and not the salt solution played the major rôle in producing the changes observed.

The aseptic meningeal reaction that follows the intrathecal injection of metaphen or mercurochrome is probably the result of an injury to the meninges causing increased permeability of the blood vessels with exudation of erythrocytes and plasma protein, i. e., mainly albumin, into the subarachnoid space. Whether or not such a reaction in the presence of bacterial meningitis would be beneficial per se as a result of the outpouring of immune bodies along with the plasma proteins from the blood into the cerebrospinal space, which is usually devoid of such immune bodies, or as a result of a nonspecific protein reaction is open to question.

In the clinical use of antiseptics in suppurative meningitis, much more can be expected from lavage with the antiseptic than from its simple injection. The ideal method of reaching most of the subarachnoid space is by lavage from two needles inserted through trephined openings over the right and left sides of the vertex to a cisternal or lumbar needle, as has been done by Wegeforth and Essick,<sup>9</sup> Kolmer<sup>27</sup> and Stewart<sup>11</sup> in their experimental work on meningitis. We, however, have limited ourselves to lavage from the cistern to the lumbar region, as we believe this to be the route of choice in the lavage treatment for meningitis in man. We are now trying out lavage from the lumbar region to the cistern in the hope that it will be accompanied with fewer accidental deaths than cistern-lumbar lavage and also because, as suggested by Pollock and Favill,<sup>29</sup> it may be a better method of reaching the cortical subarachnoid space than by cistern-lumbar lavage.

Because mercurochrome in bactericidal concentrations is lethal, its employment as an intrathecal disinfectant is contraindicated. Its "non-injurious affinity for body tissues" does not apply to the tissues comprising the central nervous system. In view of the fact, however, that metaphen is from 25 to 100 times more bactericidal than mercurochrome, we have not discarded it, as we have mercurochrome. As far as we know, metaphen has not been used previously to any extent in the treatment for meningitis. Kolmer<sup>27</sup> mentioned having used it in this disease

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28. Weed, L. H., and Wegeforth, P.: *J. Pharmacol. & Exper. Therap.* **13**:317, 1919.

29. Pollock, L. J., and Favill, J.: *The Human Cerebrospinal Fluid*, New York, Paul Hoeber, 1924, p. 475.

with disappointing results, but he stated that his experience with it had been limited.<sup>30</sup> We believe that it is worthy of further trial in meningitis, for it can be introduced in bactericidal strength in subtoxic doses.

#### SUMMARY

Subthecal punctures and injections in the dog are attended by difficulties and frequently by injuries to the animal. With the proper technic, however, these may usually be circumvented.

The maximum subtoxic dose of mercurochrome by intracisternal injection was between 0.07 and 0.11 mg. per kilogram; the minimum lethal dose was between 0.11 and 0.15 mg. per kilogram. By intraspinal injection, the toxicity was less, the maximum subtoxic dose being between 0.17 and 0.22 mg. per kilogram.

The maximum subtoxic dose of metaphen by intracisternal injection was between 0.10 and 0.12 mg. per kilogram; the minimum lethal dose was between 0.16 and 0.18 mg. per kilogram. By intraspinal injection, the toxicity was less, the maximum subtoxic dose being between 0.14 and 0.22 mg. per kilogram.

Lavage from the cistern to the lumbar region with a 1:1,000 solution of mercurochrome was fatal to dogs, but lavage with a 1:25,000 solution of metaphen was well borne.

Mercurochrome was bactericidal in dilutions up to 1:1,000 under the same conditions in which metaphen was bactericidal in dilutions up to 1:50,000.

After the intrathecal injection of either mercurochrome or metaphen, the following changes occurred in the cerebrospinal fluid: (*a*) increased pressure; (*b*) increased number of cells, first of erythrocytes and later of polymorphonuclear leukocytes and lymphocytes, the former predominating; (*c*) increased protein content, mainly due to albumin to such an extent that the fluid coagulated on standing.

An aseptic serofibrinopurulent leptomeningitis followed the intrathecal injection of either mercurochrome or metaphen.

Mercurochrome should not be administered intrathecally clinically, because sublethal doses are not bactericidal.

Metaphen deserves further investigation as an intrathecal disinfectant, for not only does it have a larger margin of safety than mercurochrome, but it is bactericidal in sublethal doses.

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30. Kolmer, J. A.: Personal communication to the authors.

# CONGENITAL ATRESIA OF THE ESOPHAGUS WITH TRACHEO-ESOPHAGEAL FISTULA

REPORT OF EIGHT CASES \*

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According to Mackenzie,<sup>1</sup> the first recorded case of congenital atresia of the esophagus was that of Durston in 1670. In 1861, Hirschsprung<sup>2</sup> collected the reports of 10 cases from the literature and added 4 of his own. In 1884, Mackenzie<sup>1</sup> collected the reports of 62 cases and reported a new case. In 43 of the cases recorded to that time a tracheo-esophageal fistula was present. The most complete review of the literature was that of Plass,<sup>3</sup> who in 1919 collected the reports of 136 cases of esophageal atresia. A careful search of the literature has revealed 85 additional cases of esophageal atresia reported since Plass' review; 61 of these were associated with tracheo-esophageal fistula, and 14 were not, as shown by postmortem examination. Since an autopsy was not permitted in 10 cases, the presence or absence of a fistula was not fully established in all of these cases, although the clinical findings pointed to its presence in the majority of these instances. The presence of the atresia, however, was definitely diagnosed by the characteristic clinical picture. Thus, to date, including the 8 cases described here, there are 255 recorded cases of congenital esophageal atresia, in 205 of which a tracheo-esophageal fistula was present, and in 40 of which it was absent.

The aforementioned number of cases cannot, of course, be taken as the number of cases that have occurred. Authors are at great variance in their conceptions of the frequency of this condition. As stated in an accompanying clinical report, the indications are that many more cases have occurred than have been reported or even diagnosed.

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\* Submitted for publication, May 22, 1931.

\* From the Division of Laboratories, Mount Sinai Hospital, New York, and the Carnegie Institution of Washington, Department of Embryology, Baltimore.

1. Mackenzie, Morell: *A Manual of Diseases of the Throat and Nose*, New York, William Wood & Company, 1884, vol. 2, p. 149.

2. Hirschsprung, cited by Mackenzie (footnote 1).

3. Plass, E. D.: *Congenital Atresia of the Esophagus with Tracheo-Esophageal Fistula*, Johns Hopkins Hosp. Rep. **18**:259, 1919.

## EIGHT CASES

The symptoms and the clinical aspects of this anomaly in 8 cases may be found in the accompanying report. A brief summary of the characteristic symptoms is as follows:

The infant is usually well nourished and well developed at birth, and with the exception of the presence of a large amount of mucus in the mouth and pharynx and often in the trachea, no abnormalities are suspected. Occasionally this may cause asphyxia. The most characteristic and constant symptom is the typical episode that attends each attempt at feeding. The infant accepts fluids readily enough, but after a few swallows stops, ceases to breathe, becomes cyanotic and regurgitates a frothy discharge through the nose and mouth. A period of almost lifeless relaxation may follow this episode, but the child almost always recovers and repeats the performance, if given the opportunity. The weight falls steadily, and the infant rapidly becomes dehydrated. The average loss of weight before death is from 25 to 40 per cent. Physical examination a short time after birth may reveal little except a whitish, frothy mucoid material in the nasopharynx. Depression of the anterior fontanel and a dry, adherent skin may be seen later as dehydration becomes marked. The epigastrium is often distended, and percussion may reveal tympany of the stomach, a sign of the presence of the tracheo-esophageal fistula. For a few days the stools are normal in quantity and are typical meconium. Later they become scant or absent and are composed merely of bile-stained mucus—the stools that characterize starvation. The urine is scanty and concentrated, and early suppression is seen. The temperature is usually raised by the advent of inanition fever after from two to three days, and may be influenced by the bronchitis and bronchopneumonia that commonly complicate the clinical picture. The temperature may, however, remain normal or become subnormal. The diagnosis is confirmed by the passage of a catheter, which meets an obstruction at about from 10 to 12 cm. from the alveolar ridges. Roentgen examination after the introduction of a barium mixture into the pharynx reveals a dilated culdesac reaching usually to about the fourth thoracic vertebra.

CASE 1.—B. D., a boy, died four days after birth. Postmortem examination revealed a poorly nourished infant on whom a gastrostomy had been performed. The pharynx led into a widely dilated esophagus, which was completely closed at a point 1 cm. above the bifurcation of the trachea. From this point on, the esophagus was nonexistent as a patent tube, but continued as a ridge in the form of a longitudinal thickening on the posterior wall of the trachea. It continued in this fashion to a point a little above the bifurcation of the trachea, where it once more emerged as a patent tube. Its lumen at this point communicated with the trachea in a manner resembling the entrance of the ureter into the bladder. It joined the stomach in a normal fashion. A widely patent ductus arteriosus was present, as well as a patent foramen ovale (fig. 1 *A* and *B*).



CASE 2.—B. H., a boy, died six days after birth. Postmortem examination revealed a fairly well developed and well nourished infant. The upper third of the esophagus terminated in a short fibrous cord which was firmly attached to the posterior wall of the trachea near the bifurcation. The lower two thirds of the esophagus was found to open into the lumen of the trachea just below this point and above the bifurcation. The heart was enlarged, owing to a dilatation of the right auricle and right ventricle. A widely patent ductus arteriosus was present. The foramen ovale was closed. Aspiration bronchopneumonia and malposition of the transverse colon were also present.

CASE 3.—K. I., a boy, died six days after birth. Postmortem examination revealed the upper part of the esophagus to be dilated and to end as a blind pouch about 4 cm. below the hyoid bone. It was markedly hypertrophied. No cord was seen extending from this blind end caudally. The trachea at its bifurcation was

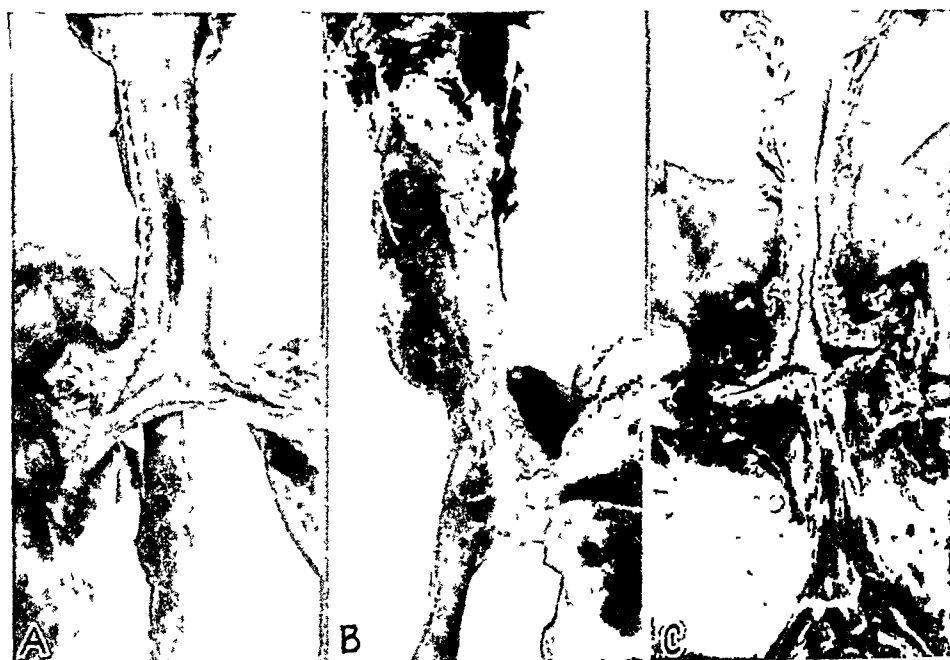


Fig. 1.—Specimens showing congenital atresia of the esophagus with tracheo-esophageal fistula: *A* and *B*, case 1; *C*, case 3.

seen to divide into three divisions, two lateral cartilaginous ones, which were evidently the bronchi, and a mesial noncartilaginous one, which was the lower end of the esophagus and extended to the stomach. The stomach was small and filled only with a mucoid secretion. No other abnormality was present in the gastrointestinal tract. The heart was entirely normal, except for a huge patent foramen ovale, measuring 1.5 cm. in diameter. It appeared almost like a complete interauricular septal defect. Bronchopneumonia was present (fig. 1 *C*).

CASE 4.—B. W. died five days after birth. Postmortem examination revealed the upper portion of the esophagus ending as a blind pouch about 4 cm. long. This blind end was still attached to the lower portion of the esophagus by a very fine film of tissue. The lower portion of the esophagus entered the trachea about 2 cm. above the bifurcation. A patent foramen ovale was present, and the lungs were partially atelectatic.

CASE 5.—H. G., a boy, died seven days after birth. Postmortem examination revealed the upper third of the esophagus ending in a culdesac which was attached by a thin string of fibrous tissue to the posterior peritracheal tissue. The lower two thirds of the esophagus was in direct communication with the trachea just at its bifurcation. The other pathologic findings included a patent foramen ovale and bronchopneumonia.

Drs. Lederer and Wilson of the Jewish Hospital of Brooklyn permitted us to study and report the following 3 cases.

CASE 6.—B. W., a boy, died nine days after birth. Postmortem examination revealed the upper part of the esophagus ending in a blind sac about 6 mm. in diameter and 5 cm. in length. For a distance of 1.5 cm., it extended as a cord-like structure, which became continuous with the distal 6 cm. of the lower part of the esophagus. The latter was of normal caliber and structure and communicated with the trachea by a slitlike fistulous connection, situated about 0.5 cm. above its bifurcation. The foramen ovale was slightly patent, and the lungs were somewhat atelectatic.

CASE 7.—B. K., a boy, died six days after birth. Postmortem examination revealed the upper portion of the esophagus as a dilated, hypertrophied sac about 4 cm. in length. It continued as a cord along the posterior aspect of the trachea to about 1 cm. above its bifurcation, where it again became patent, communicated with the trachea and then continued normally to the stomach. The other abnormal findings included the absence of the left kidney and ureter, a patent foramen ovale and patent ductus arteriosus and bronchopneumonia.

CASE 8.—B. L., a boy, died eight days after birth. Postmortem examination revealed the upper fourth of the esophagus ending blindly as a dilated pouch. Its lumen was twice that of the lower part of the esophagus. The two portions were separated by a distance of 1 cm., and no connecting cord was present. The fistulous opening was just above the bifurcation. The skin was dry and wrinkled. The foramen ovale was closed, but the ductus arteriosus was patent. Bronchopneumonia was present.

A summary of the histology of the first 4 cases is presented later.

#### GROSS PATHOLOGY

As has been pointed out several times, the gross picture of the anomaly is markedly constant. Reference to the appended photographs of two of the specimens demonstrates this striking change clearly. The upper portion of the esophagus begins normally, but ends as a blind sac at varying distances below the larynx. Fischer (Henke and Lubarsch<sup>4</sup>) stated this length to be between 1.5 and 2 cm. Plass<sup>3</sup> gave the average length as about 3.4 cm. on the basis of 72 cases in 5 of which it measured from 1 to 1.9 cm. and in 9, 5 cm. or over, with the measurements of the others ranging between. The average length in the cases reported here agrees with that of Plass.<sup>3</sup> The pouch is almost always hypertrophied and uniformly dilated to a diameter of

4. Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol. 1, p. 84.

about 1 cm. or more, whereas the normal width of the esophagus in the new-born infant is about from 4 to 6 mm. This is true of practically all of the specimens studied here. Many believe the hypertrophy and dilatation to be the result of ineffectual attempts of the fetus to swallow amniotic fluid. Ballantyne,<sup>5</sup> Brigham,<sup>6</sup> Meyer<sup>7</sup> and Scheurer<sup>8</sup> noted the association of hydramnion with atresia of the esophagus and believed the hydramnion to be the result of the inability of the fetus to swallow and absorb the amniotic fluid. Plass<sup>3</sup> noted the association in 9 of 136 cases, but believed it to be mere coincidence. Others believe the hypertrophy to be due to an abnormal growth stimulus. Giffhorn<sup>9</sup> stated that since the posterior wall of the esophagus is fixed while growth continues, the entire blind sac must be pulled out in the manner that a traction diverticulum is formed, resulting in hypertrophy and dilatation.

The lower portion of the esophagus makes a fistulous connection with the trachea at a varying distance above the bifurcation of the latter into the bronchi, the average distance being about 0.5 cm.; in 2 of the cases reported here the fistula was at the bifurcation; in 1 case, just above the bifurcation; in 2 cases, 0.5 cm. above, and in 3, 1.0, 1.5 and 2 cm. above, respectively. Of 96 cases cited by Plass,<sup>3</sup> 94 showed the distance to be within this same range; in the remaining 2, a fistulous communication with a bronchus was found. A review of the macroscopic description in about 80 cases reported since Plass' review has shown that in these cases this distance fell within the same range. In 5 cases, a communication with a bronchus was noted; in 4 cases (Baranger,<sup>10</sup> Ellerbroek<sup>11</sup> and Hamm et al.<sup>12</sup>) the right bronchus was concerned, and in 1 the left bronchus. In the last case no actual fistula was present, and the esophagus continued as a cord. Sencert<sup>13</sup> stated that when the fistula is above the bifurcation, there are rarely concomi-

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5. Ballantyne, J. W.: *Manual of Antenatal Pathology and Hygiene*, Edinburgh, W. Green & Sons, 1904, p. 462.

6. Brigham, R. O.: *Atresia of the Esophagus*, *Am. Med.* **35**:297, 1929.

7. Meyer, H.: *Esophagusatresie und Hydramnion*, *Zentralbl. f. Gynäk.* **53**: 1562, 1929.

8. Scheurer, D.: *Ein Fall von kongenitaler Oesophagusatresie mit Oesophago-Trachealfistel*, *Schweiz. med. Wchnschr.* **9**:1079, 1928.

9. Giffhorn, H.: *Beitrag zur Aetiologie der kongenitalen Atresia des Oesophagus mit Oesophago-trachealfistel*, *Virchows Arch. f. path. Anat.* **192**:112, 1908.

10. Baranger, A.: *Curieuses malformations des voies digestives supérieures*, *Arch. internat. de laryng.* **34**:1077, 1928.

11. Ellerbroek, N.: *Ein Beitrag zur Oesophagusatresia*, *München. med. Wchnschr.* **69**:591, 1922.

12. Hamm, A., et al.: *Un cas d'atrésie congénitale de l'oesophage*, *Bull. et mêm. Soc. anat. de Paris* **94**:631, 1924.

13. Sencert, cited by Rogier, P.: *Quelques considérations sur deux nouveaux cas d'imperforation de l'oesophage*, *Thèse de Paris*, 1927, vol. 59.

tant malformations, while when it is at the same height or in the right bronchus, malformations of the heart and great blood vessels are the rule. No evidence of such an association has been encountered in a review of the literature. The fistulous opening has a characteristic crescent shape and has been aptly described as resembling the opening of the ureter into the bladder. This tracheal opening is always smooth, the mucous membranes of the tubes being continuous. The muscular walls frequently blend without any demarcation. Occasionally a groove-like depression may be seen running up the posterior membranous portion of the trachea from the region of the fistula. The lower portion of the esophagus seems to spring as a direct continuation from the muscular posterior wall of the trachea, beginning as a narrow tube measuring about from 4 to 7 cm. in length and gradually increasing in size to its normal caliber. It opens into the stomach in a normal fashion. In a case presented here, the lower portion of the esophagus measured 3 cm. in diameter at the fistulous end and 6 mm. at the cardiac end, and in another it measured 4 mm. at the fistulous end and 7 mm. at the cardiac end. According to Plass,<sup>3</sup> rudimentary tracheal cartilages have been found in the fistulous portion of the esophagus.

Occasionally, an overlapping of the two segments has been noted. In twelve of the fourteen specimens examined by Keith,<sup>14</sup> who collected them from medical schools and hospitals in England, the fundus of the upper segment overlapped the origin of the lower segment, and there was usually a greater or lesser degree of union between the musculature of the two portions of the esophagus and that of the trachea. An overlapping was noted in two of the specimens presented here; in one it was slight, and in the other marked. In the latter, the distance of overlapping was 1.7 cm. Keene<sup>15</sup> reported a similar case. If one does not accept mechanical factors as playing an important rôle in an elongation of the upper culdesac, this type of variation speaks against the theories concerning the development of the anomaly which have to do with pressure atrophy. Usually, however, the two segments of the esophagus are separated by a definite distance of about 1 cm. The maximal distance is about from 4 to 5 cm. In a case reported by Levys<sup>16</sup> the defect was 5 cm. The separation is usually bridged by a fibromuscular cord. No cord was present in two of the specimens presented here, and in one existed only as a fine film of tissue. Fischer (Henke and Lubarsch<sup>4</sup>) stated that a cord was present in 53 of 73 cases in which an exact study was made.

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14. Keith, Arthur: Constrictions and Occlusions of the Alimentary Tract of Congenital or Obscure Origin, *Brit. M. J.* **1**:301, 1910.

15. Keene, R.: A Case of Irregular Separation of the Trachea from the Esophagus, *St. Barth. Hosp. J.* **28**:155, 1920-1921.

16. Cited in Henke and Lubarsch (footnote 4).

The question of the occurrence and type of congenital anomalies associated with this condition is of interest and importance. Several authors have stated that the frequent association of other anomalies incompatible with life makes surgical intervention for the atresia of the esophagus inadvisable. On the basis of cases recorded to date, this is not true and is an obstacle to advance in the treatment of infants with this condition. Reynolds and Morrison<sup>17</sup> stated that associated malformations are uncommon. The careful review of Plass,<sup>3</sup> however, shows that they are somewhat frequently present. Thus they were found in 59 of 94 cases in which the presence or absence of other anomalies was noted (patent ductus arteriosus and foramen ovale were not classed as anomalies), but few of the associated malformations were in themselves incompatible with life. By far the most common was atresia ani, noted in 24 cases. In a small proportion of the cases recorded since Plass' time, associated anomalies were noted, only one of which would have been fatal. The anomalies were: atresia duodeni, deformities of the hand, absence of the left kidney, double uterus and harelip. In 3 of the 8 cases reported here, other, relatively harmless abnormalities were present. They were absence of the left kidney, malposition of the transverse colon and slight deformity of the hand. The marked frequency of the presence of patent foramen ovale and a patent ductus arteriosus was noted. Every specimen presented at least one, and three had them both combined.

A presentation of the gross pathology would not be complete without reference to the frequent pulmonary complications seen with this anomaly. With the tracheo-esophageal fistula and the rapid inanition that sets in as a result of the atresia of the esophagus, it can be readily understood why tracheobronchitis and bronchopneumonia so commonly hasten the death of the infant. Thus aspiration bronchopneumonia was present in 5 cases and atelectasis in 2.

#### HISTOLOGY

As a general rule, gross specimens have been kept on exhibition. One is reluctant to section specimens showing grossly so striking an anomaly. As a result there have been few contributions to the histology of the malformation. Trillat and Pigeaud<sup>18</sup> reported that their studies showed both portions of the esophagus to be histologically normal. However, most others who made microscopic examinations reported varying types of histologic changes. Ballantyne<sup>5</sup> stated that normal

17. Reynolds, R. P., and Morrison, W. W.: Congenital Malformations of the Esophagus, *Am. J. Dis. Child.* **21**:339, 1921.

18. Trillat and Pigeaud: Un cas de communication entre l'oesophage et la trachée-rohres, *Bull. Soc. d'obst. et de gynéc.* **17**:75, 1928.

stratified squamous epithelium was found in both the upper and the lower portions of the esophagus, and cited Luschka as having found striped muscle in the upper portion and only unstriped muscle in the lower. Fourteen specimens were collected and examined by Keith.<sup>14</sup> He believed that the break in the esophagus occurred at what he considered was the junction of two parts of different origin. He considered that the upper part was pharyngeal. It was almost entirely composed of striated muscle fibers. He considered the lower part as having been derived from the primitive esophagus. This was composed of non-striated muscle fibers. These histologic observations are, of course, a confirmation of Ballantyne's<sup>5</sup> statement. Latz<sup>19</sup> reported a case in which the muscle bundles of the upper part of the esophagus not only were composed exclusively of the striated type, but were all longitudinal in their course. The musculature of the lower portion was composed of smooth muscle.

A few histologic studies have shown abnormalities in the structure of the trachea, predominantly in the region of the fistula. Ladwig<sup>20</sup> reported a case in which the lower portion of the esophagus was seen to continue in the posterior wall of the trachea. Thick circular and longitudinal layers of muscle and glands, similar to those found in the esophagus, were found in the trachea. In this case he could demonstrate stratified squamous epithelium continuing in the posterior wall of the trachea, and its extension to the thyroid cartilage. He therefore considered the posterior wall of the trachea to be in the nature of an esophageal wall. Konopacki<sup>16</sup> also found squamous epithelial cells in the mucosa of the trachea in the region of the fistula. He also found an abnormal thickness of the musculature of the trachea, which contained several cross-striated fibers. Gutmann<sup>21</sup> stated that he found fibers of the posterior wall of the esophagus continuing for a distance in the anterior wall of the trachea.

*Summary of the Microscopic Structure of Four Specimens Reported Here.*—The upper culdesac was of a uniform structure in the four cases examined. The epithelium was of the normal stratified squamous variety. The muscularis mucosa was irregularly slightly hypertrophied. Occasionally, it was seen to be present as a very thin layer of smooth muscle, and it was actually absent in a region in one of the sections. The submucosa was present as a definite layer in the structure of the wall and contained few mucous glands. The musculature showed the most marked

19. Cited in Henke and Lubarsch (footnote 4).

20. Ladwig, A.: Ein bemerkenswerter Fall von Missbildung des Oesophagus-Tracheal-Rohres, *Centralbl. f. allg. Path. u. path. Anat.* **31**:613, 1920.

21. Gutmann, S.: Ueber einen Fall von Oesophagusatresie und Oesophago-Trachealfistel, *Frankfurt. Ztschr. f. Path.* **9**:459, 1912.

changes. It was regularly considerably hypertrophied and appeared normal in thickness only when the upper sac was considerably dilated. Although it was in general composed of an outer longitudinal and an inner circular layer, this arrangement was frequently distorted, and in one section it was reversed for a short distance. The hypertrophy of the musculature was accompanied by overdevelopment of Auerbach's plexus. The musculature was composed mostly of striated skeletal muscle, especially the outer longitudinal layer. As one would expect, the proportion of smooth muscle increased in amount as one proceeded caudadly. This was true especially of the inner layer. The outer longitudinal layer, however, generally consisted of the striated type to the very tip, with the exception of the anterior portion, which consisted mostly of smooth muscle. The anterior wall was seen to fuse with the smooth muscle of the posterior membranous wall of the trachea. A large amount of fibrous tissue was present within the musculature, being so marked in areas as to interrupt completely the course of the muscle fibers.

With the exception of its fistulous end, the lower portion of the esophagus was normal in structure. At this site, the esophagus had essentially a tracheal structure. The mucosa in three of the specimens was of the tracheal type and in one was of an undifferentiated type. In every instance, mixed glands were seen to be dispersed among the muscle bundles of the esophagus in this region. Study of sections demonstrated that the normal musculature of the lower portion of the esophagus became irregular as it approached the trachea. The arrangement of normal esophageal musculature, which is composed of inner circular and outer longitudinal layers, became irregular as it approached the region of the fistula and continued as a longitudinal layer continuous with the smooth muscle of the posterior membranous wall of the trachea. Other than the fistulous opening, no abnormalities were noted in the structure of the trachea.

Because of the presence of tracheal structures in the upper end of the lower portion of the esophagus and the change in musculature of the upper sac from the striated to the nonstriated type in its anterior wall proximal to the trachea it was thought that both sections of the esophagus shared in the tracheal structure. The musculature of the upper sac was interpreted as being similar to that of the membranous wall of the trachea to which it was fused. However, examination of apparently normal esophagi and tracheae in four infants of approximately the same age presented this alternation of smooth and striated muscle to almost the same degree. Nevertheless, as shown in the previous paragraph, the fistulous end of the lower segment of the esophagus had a tracheal structure. This observation was contrary to those reported previously, in which esophageal elements were found in the trachea and no tracheal

elements in the esophagus. If one reviews the histologic descriptions in all the reports, one notices that there has been a lack of uniformity in the microscopic structure of the fistulous site. Even the trachea has been reported to show abnormalities in its histologic structure.

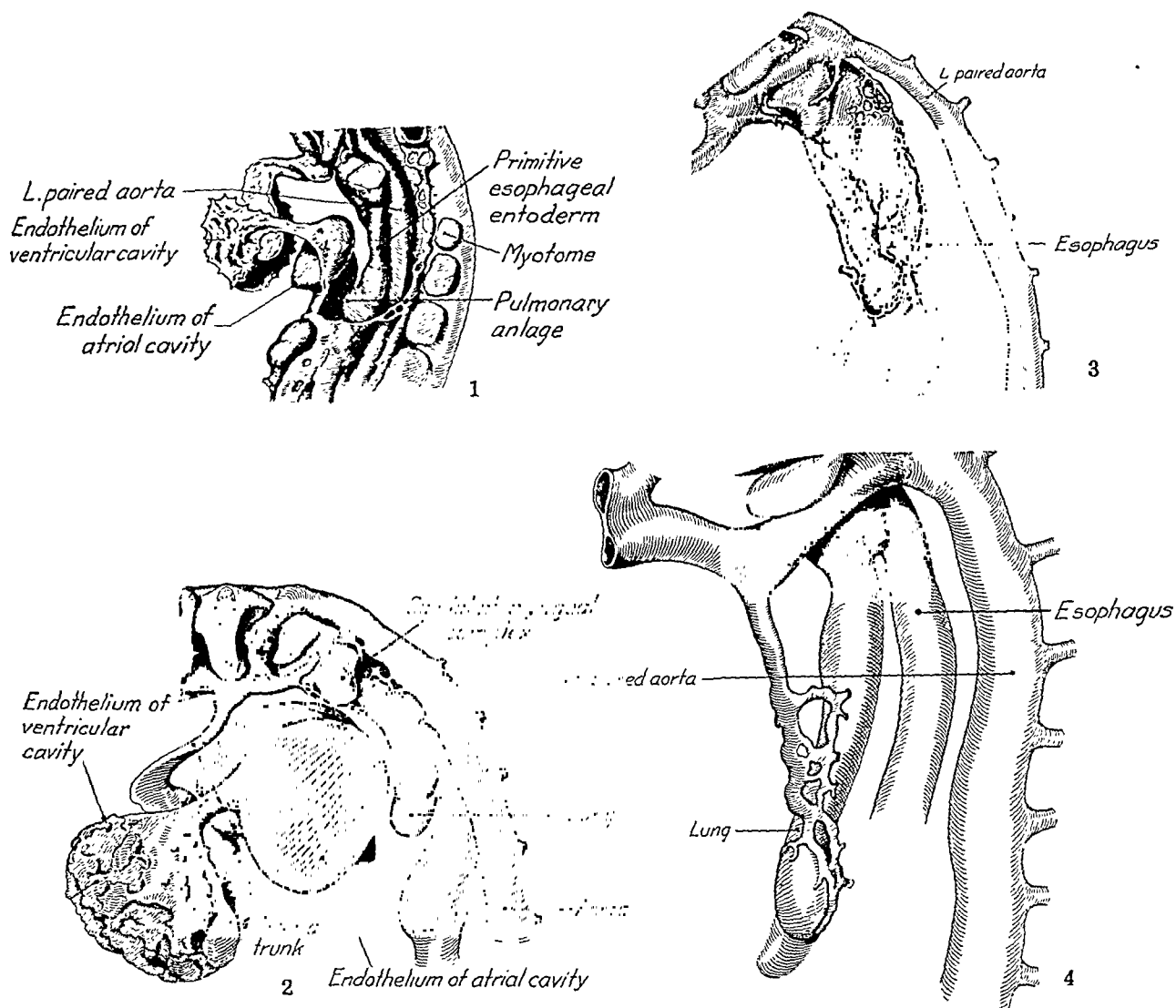


Fig. 2.—Embryos of 3, 4, 6 and 11 mm., illustrating the stages in the normal development of the esophagus and trachea (modified from Congdon, E. D.); Contrib. Embryol., Carnegie Inst., Washington **14**:47, 1922.

#### NORMAL DEVELOPMENT OF ESOPHAGUS AND TRACHEA FROM THE PRIMITIVE FOREGUT

A brief résumé of the normal development of the esophagus and trachea from the primitive foregut may aid in the interpretation of the various theories advanced. In the 3 mm. embryo (fig. 2) the first anlage of the respiratory system (pulmonary anlage) appears caudal to the pharyngeal pouches as a ventral rounded mass of entoderm. In



the 4 mm. embryo growth of the anlage has resulted in caudal prolongation and in the formation of two lateral longitudinal grooves, of which the corresponding internal elevation is the anlage of a septum. In this stage the trachea is seen to be already partially separated from the esophagus. Reference to the diagrammatic figure will illustrate the appearance of a cross-section of this region at this stage. The separation is more definite in the 5 mm. embryo and practically complete in the 11 mm. embryo. To many authors, the apparent cephalad growth of the ventral transverse groove between the lung anlage and the esophagus, along with the deepening of the longitudinal lateral grooves, constitutes a tracheo-esophageal septum which ultimately effects a complete separation in a cephalad direction.

#### GENESIS OF THE ANOMALY

Many theories have been advanced to explain the congenital anomaly considered here. Luschka<sup>22</sup> and independently Klebs<sup>23</sup> held that fetal inflammation or intra-uterine trauma played the important rôle. The marked constancy of the malformation makes any theory that has to do with inflammatory or traumatic factors seem unlikely. In the theories proposed by Robin,<sup>24</sup> Leven<sup>25</sup> and Regnier,<sup>26</sup> the upper portion of the esophagus or the entire esophagus is considered as ectodermal. This it most certainly is not. A study of early embryos demonstrates the epithelium of the entire esophagus to be derived from entoderm. Klebs,<sup>23</sup> and later Kraus,<sup>27</sup> advanced the conception that too much of the original formative material was used up in the development of the trachea, bronchi and lungs, so that an atresia of the esophagus resulted. Shattock's<sup>28</sup> explanation is somewhat similar. He believed that the posterior wall occasionally participated in the formation of the respiratory apparatus and was brought forward, thus narrowing the lumen. This forms the basis for his explanation of the development of the anomaly. The remaining theories are those that are most widely accepted, and may be grouped under three headings.

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22. Luschka, cited by Zeit, F. R.: Congenital Atresia of the Esophagus, *J. M. Research* **22**:45, 1912-1913.

23. Klebs, cited by Zausch, P.: Ein Fall von Oesophagusatresie und Oesophago-Trachealfistel, *Virchows Arch. f. path. Anat.* **234**:94, 1921.

24. Robin, cited by Rogier (footnote 13).

25. Leven, cited by Gutmann (footnote 21).

26. Regnier, cited by Trillat and Pigeaud (footnote 18).

27. Kraus, cited by Ysander, F.: Zur Frage der Genese der Oesophagus-atresien, *Upsala läkaref. förh.* **30**:195, 1924.

28. Shattock, S. G.: Congenital Atresia of the Esophagus, *Tr. Path. Soc. London* **41**:87, 1890.

*Theories Dealing with Retardation of Esophageal Development at a Time When the Esophagus Is Still Solid.*—Kreuter<sup>29</sup> stated that to the fifth week the esophagus is a tube. From this period on, intensive epithelial proliferation results in the lumen being reduced to a slit. The most marked proliferation appears at the region of the bifurcation. Later vacuolization of the epithelium causes a dissolution of the atresia, so that finally a permanent lumen is formed. Permanence of the early embryonal phase in which the esophagus is solid causes the atresia. The epithelium perishes and is replaced by connective tissue. This theory was apparently corroborated by Reese,<sup>30</sup> who stated that temporary closure of a part of the anterior region of the digestive tract is commonly observed in the various classes of lower vertebrates in early embryonal life. The work of Schridde<sup>31</sup> on fifty human embryos constitutes a direct contradiction to Kreuter.<sup>29</sup> Schridde found the esophagus to have a lumen at all periods of its development. There are no vacuoles; what appear to be vacuoles are epithelial bridges due to epithelial proliferation in circumscribed regions. In the region where the atresia is formed, the epithelial bridges are not present. He believed that Kreuter's conception was a misinterpretation of the facts. Further, as pointed out by Forssner,<sup>32</sup> a study of embryos demonstrates that the separation of the trachea from the esophagus occurs when the embryo is at the early stage of about from 4 to 5 mm., whereas the so-called epithelial obliteration occurs at the much later stage of about 20 mm. It is unlikely that the anomaly develops at more than one stage in the formation of the trachea and esophagus.

*Theories Concerning Deviation of the Tracheo-Esophageal Septum.*—According to Keith and Spicer,<sup>33</sup> the lateral tracheo-esophageal ridges and folds normally proceed horizontally backward so as to meet between the pulmonary buds and the esophagus and so divide the primitive esophagus into a dorsal and a ventral portion. To produce this anomaly they must proceed obliquely backward and dorsalward so as to meet on the dorsal wall of the foregut. Ribbert<sup>16</sup> also believed that the lower part of the separating septum becomes attached to the posterior wall of the foregut, thus causing the atresia. Konopacki and Hoffman<sup>34</sup> advanced

29. Kreuter, cited by Schridde, H.: Ueber Epithelproliferationen in der menschlichen Speiseröhre, Virchows Arch. f. path. Anat. **191**:178, 1908.

30. Reese, A. M.: The Occlusion of the Oesophagus and Trachea in Crocodilla and Snakes, Am. J. Anat. **37**:195, 1926.

31. Schridde, H.: Ueber Epithelproliferationen in der menschlichen Speiseröhre, Virchows Arch. f. path. Anat. **191**:178, 1908.

32. Forssner, cited by Schmitz, J. A.: Ueber de formate Genese der Oesophagus Missbildungen, Virchows Arch. f. path. Anat. **247**:278, 1923.

33. Keith, A., and Spicer, J. E.: Three Cases of Malformation of the Tracheo-Esophageal Septum, J. Anat. & Physiol. **41**:52, 1906-1907.

34. Konopacki and Hoffman, cited in Abt, Isaac A.: Pediatrics, Philadelphia, W. B. Saunders Company, 1924, vol. 3, p. 407.

a similar theory. They assumed the lateral crests which should separate the respiratory apparatus from the esophagus to deviate from the normal pathway in an oblique course. To Zeit,<sup>35</sup> arrested development of the lateral ridges, which unite incompletely in the midline, explains the fistula and a "faulty anlage" the atresia. Lewis<sup>36</sup> believed that the lower portion of the tracheo-esophageal septum fails to develop, thus leaving the esophagus in communication with the lower part of the trachea. He called attention to a lateral furrow of the wall situated externally on either side of the esophagus caudad to the ventral pulmonary anlage and presenting a corresponding internal elevation. This same depression was seen in reconstructed models of the entoderm of 4 mm. embryos in the collection of the Carnegie Institution of Washington. It is so situated that if the walls of the esophagus should coalesce along this groove a ventral portion would be cut off, communicating freely with the trachea near its bifurcation. The part of the esophagus dorsal to this groove has a narrower lumen than the ventral part, and to produce the anomaly this portion must become occluded. Giffhorn<sup>9</sup> advanced two explanations. One is similar to that of Keith and Spicer,<sup>33</sup> and the second is based on a pushing forward of the posterior wall to meet the lateral folds in a manner similar to that explained in Shattock's<sup>28</sup> theory. As stated, Ladwig<sup>20</sup> found esophageal elements in the posterior wall of the trachea and therefore considered the posterior wall of the trachea as the anterior wall of the esophagus, and also held that the fact that the separation of the trachea and the esophagus exists in the upper portion and not in the lower meant that separation proceeds caudad, not cephalad. His histologic observations will be interpreted in a different manner in a later discussion. That normal separation proceeds in a cephalad direction has been shown here, and there is no evidence that Ladwig's interpretation is true.

*Theories Concerning Changes of Tension.*—Theories concerning changes of tension are among those most recently advanced and appear almost entirely in the German literature. Zausch,<sup>37</sup> a pupil of Beneke, advanced his mechanical theory. To him, the direction of pressure exerted by the heart anlage determines the type of anomaly produced. Thus, if it pushes in a cephalad direction, it prevents proper closure of the palatal clefts and results in cleft palate. If it is directed more pharyngealward, the pressure would act on the "narrow isthmus" of the esophageal anlage which overlies the heart, resulting in esophageal atresia and tracheo-esophageal fistula. In a case that he observed, an

35. Zeit, F. R.: Congenital Atresia of the Esophagus, J. M. Research **22**:45, 1912-1913.

36. Lewis, cited by Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1910, vol. 2, p. 312.

37. Zausch, P.: Ein Fall von Oesophagusatresie und Oesophago-Trachealfistel, Virchows Arch. f. path. Anat. **234**:94, 1921.

associated bifid uvula was interpreted as a palatal cleft, and both were conceived to be caused by pressure of the heart anlage and marked cervical curvature. Schmitz<sup>38</sup> also considered the primary cause to be pressure from the heart anlage. He called attention to the fact that the primitive foregut consists of only one layer of cells dorsally and three layers ventrally, with undifferentiated mesenchyme around it. The posterior wall not only is subject to the most tension, but is also the least resistant. If abnormal pressure continues, the posterior wall ruptures. The upper end becomes dislocated cranially, and the caudad end is kept in its original position by the heart anlage acting like a wedge. The epithelial cells in the ruptured end regenerate, but they cannot produce union, and hence atresia results. The pressure of the heart on the lower end leads to fusion of its posterior wall with the longitudinal lateral crests and results in a fistula.

Ysander<sup>39</sup> described an 8 mm. embryo of a monster of the type thoracopagus tetrabrachius in which there was but one stomach and one heart anlage, while the esophagus and the respiratory apparatus were duplicated identically. Each presented the typical esophageal atresia with tracheo-esophageal fistula seen in postnatal cases. This is the youngest embryo reported showing the anomaly, the only other being an 18.1 mm. embryo in the Harvard collection, which is single and similar to the postnatal type. Ysander explained the esophageal anomaly in a manner somewhat similar to that of Zausch<sup>37</sup> and Schmitz.<sup>38</sup> The foregut is bent in a curve by the large heart complex. The strain is put mostly on what he terms the periphery. This evidently is the dorsal wall of the primitive tube at about the midregion. This causes a scarcity of formative mass in this region, which results in a relative shifting of the lateral crests dorsalward, causing an atresia above, and a disappearance of the esophageal tube at the point of strain. The esophageal portion not only has lost the possibility of forming a tube here, but remains in communication with the trachea. Anders<sup>40</sup> agreed to these explanations assigning the important rôle to pressure.

According to several authors, pressure of adjacent arteries plays an important rôle in the formation of the anomaly. Thus the three cases presented by Keith and Spicer<sup>33</sup> showed a deep origin of the right subclavian artery. This abnormal artery crossed to the right side between the two parts of the esophagus. The right dorsal aorta has

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38. Schmitz, J. A.: Ueber die formale Genese der Oesophagusmissbildungen, *Virchows Arch. f. path. Anat.* **247**:278, 1923.

39. Ysander, F.: Zur Frage der Genese der Oesophagusatresien, *Upsala läkaref. förh.* **30**:195, 1924.

40. Anders, H. E.: Die Genese der angeborenen Stenosen und Atresien des menschlichen Darmkanals im Lichte der vergleichender Entwicklungsgeschichte, *Ergebn. d. Anat. u. Entwcklungsgesch.* **26**:343, 1925.

been said to play a part (Schmerz<sup>41</sup>), and according to Kraus (cited in Ysander<sup>39</sup>) the cases in which a muscular cord unites the two sections of the esophagus are due to fetal atrophy from pressure of arteries.

*Critique.*—In an attempt to explain the altered condition, several authors found it necessary to carry the origin of the anomaly back to an early stage, that of the embryo of from 4 to 5 mm. But even this is not early enough. Many have noted the marked constancy of the morphology of the anomaly. The eight specimens described in this paper closely resemble each other. This constancy is in itself a sign of an early fundamental change. Later changes in the embryo are more likely to result in spotty anomalies that do not conform to a definite pattern. Further, this change may even rest on a genetic basis. Mackenzie is cited as having encountered a father all of whose children, by three wives, were born showing the anomaly. This is striking and, of course, can be explained only on a genetic basis. Ysander's<sup>39</sup> interesting 8 mm. embryo in which the typical anomaly was found bilaterally points also to an early inherent change.

The importance attributed by Zausch,<sup>37</sup> Schmitz<sup>38</sup> and Ysander<sup>39</sup> to the pressure of a large cardiac anlage with or without marked cervical curvature of the primary foregut is suggestive. But this hypothesis does not conform to the anatomic facts revealed by a study of embryos of the stage in which the separation of the esophagus and trachea occurs. Firstly, a study of reconstructed models of human embryos from 3 to 5 mm. in length shows the cardiac anlage always large, but still more important is the fact that a definite distance exists between cardiac anlage and primary foregut. Secondly, only a slight curvature can be seen in that portion of the primary foregut from which the anlage for the respiratory apparatus arises ventrally, namely, the region of the later bifurcation. A more marked curvature is seen in the reconstructed entoderm in the region of the last branchial cleft, but this area is far above the one under consideration. Thirdly, as even Schmitz pointed out, it is difficult to see why the trachea should not also be more directly involved, since it is even closer to the cardiac anlage. And lastly, malformations of the heart, other than the common patent foramen ovale and ductus arteriosus, are rarely seen accompanying the anomaly, and it is difficult to conceive of a large cardiac anlage acting abnormally which yet ultimately develops into a normal heart.

It is even more unlikely that pressure from an abnormal blood vessel causes the malformation. According to Dr. Streeter's conception, as a general rule, the growth of the vascular system does not influence the development of proximal tissues, but is to the contrary influenced by them. As pointed out in Keibel and Mall's "Manual of Human Embryology,"<sup>36</sup> in the 4 mm. embryo, which is close to the stage in

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41. Schmerz, H.: Die Chirurgie. Berlin, Kirschner & Nordmann, 1928, vol. 4.

which the anomaly arises, the arteries are not near this portion of the esophagus. The dorsal aortas run on each side of the primitive entoderm in this stage, and cannot conceivably influence the development of the esophagus in a manner to produce this anomaly. It is even more unlikely that any component of the aortic arches plays a rôle, for they are ventral and considerably cranial to the site of the malformation. It is more probable that the reported association of an abnormal vascular system in this region is an effect of the anomaly and not the cause of it.

The production of an anomaly of this type rests on altered or deficient growth of cells. A consideration of abnormal planes of cleavage, purely on a mechanical basis, as is encountered in some of the theories in which emphasis is laid on a deviated tracheo-esophageal septum, disguises this concept. As has been stated, embryologic study reveals no evidence for those factors that are alleged to be responsible for a deviated or altered septum, namely, pressure of a large cardiac anlage, an exaggerated cervical curvature or an abnormal vasculature. Unless

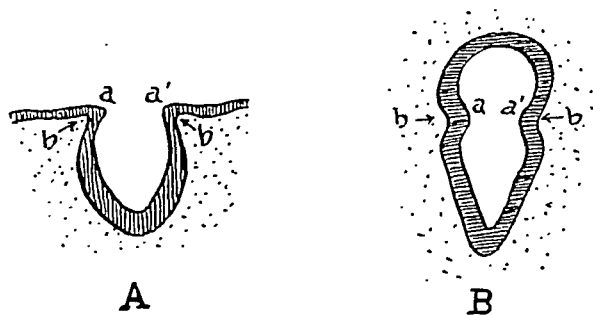


Fig. 3.—Diagrammatic representation of the neural tube of the embryonic spinal cord (A) and of the embryonic tracheo-esophageal tube (B) before the proliferation of cells that results in the closure of the former and the formation of two tubes from the latter.

concomitant primary changes are brought into play, most proponents are at a loss to explain why this abnormality of the tracheo-esophageal septum occurs. Dr. George L. Streeter suggested a simple analogy and conception of the development of the anomaly. If one first considers the formation of the common anomaly spina bifida, the explanation of the formation of the tracheo-esophageal fistula will be clearer. In the stage in which the neural tube is open, closure takes place not by the mesenchyme pushing in the entoderm at *b* and *b'* (fig. 3A), but by cellular proliferation at *a* and *a'* which later results in fusion. If for any reason there exists some deficiency in the cells at *a* and *a'* resulting in altered proliferation and nonunion, the open neural tube persists, and spina bifida results. The formation of the tracheo-esophageal fistula may be similarly considered. The two tubes, namely, a dorsal esophagus and a ventral trachea, are formed normally from one tube as the result of cellular proliferation at opposite lateral internal surfaces, *a* and *a'* (fig. 3B), and not as a result of mesenchyme pushing

the opposing lateral surfaces together at *b* and *b'*. If now for any reason a deficiency in the cells at *a* and *a'* causes altered proliferation and nonunion, a fistula between the trachea and the esophagus remains. The deficiency in the dorsal entodermal cells that were to give rise to the esophagus directly above this site may have resulted in its non-formation. Thus atresia of the esophagus results. There is some evidence that the entoderm shared in this disturbance, not only in this region but also by that over a wider area and even the surrounding mesenchyme. The abnormal histology of the tracheal and esophageal mucosa and the increased irregular musculature described under histology may be considered in this light.

If one begins with the 4 mm. embryo and traces the entoderm that is later to give rise to that portion of the esophagus dorsal to the pulmonary anlage, which is most concerned in the production of this anomaly, back through reconstructed models of earlier embryos it will be seen that in a presomite embryo the anterior termination of the neurenteric canal is in direct proximity to this portion of the entoderm. This relationship may conceivably play some rôle. In one of Dr. Streeter's embryos the neurenteric canal can clearly be seen to take an oblique course through the notochord from a dorsal caudad region to a ventral cephalad area in the entoderm. But whether the neurenteric canal plays any rôle or not, the primary change, according to the explanation presented here, is an early fundamental one that results in altered or deficient cell growth. This interpretation of the morphogenesis is not so different from those that concern altered septums and clefts. It differs markedly, however, from those in which the anomaly is ascribed to a mechanical cause. The etiologic factor is a fundamental change that rests probably on a genetic basis and not in primary concomitant associated abnormalities, such as an anomalous cardiovascular system.

#### SUMMARY

The gross structure of 8, and the microscopic structure of 4, cases of congenital atresia of the esophagus are described, with a review of the literature.

Reports on the histologic structure of this anomaly are infrequent. Microscopic examination in the cases presented revealed tracheal structures in the fistulous end of the lower segment of the esophagus. Although the gross structure of the anomaly is markedly constant, the microscopic structure, according to the histologic reports, presents marked variations.

The development of the anomaly seems to rest on an early fundamental change in the entodermal cells that are to give rise to the esophagus, and not on primary concomitant abnormalities. This change may be genetic and related to the anterior end of the neurenteric canal.

# RETICULOSIS \*

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AND

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Among the lesions of the hematopoietic apparatus, the changes involving proliferation of the reticulum cells have recently aroused increasing interest. Tumors of bone, single or multiple, deriving from endothelial cells as a matrix are well known, and as to their classification we refer to Ewing's work. There occurs, however, a rarer form of bone lesion in which the cells involved are apparently of a different type, as there is not the slightest tendency toward formation of vascular structures. These cells are also morphologically different from those described as occurring in the endotheliomas of bone. These lesions occur occasionally in connection with changes in other parts of the hematopoietic system, for example, in the lymphatic tissue, the spleen and the liver. They are accompanied in some instances by an invasion of the blood stream by immature leukocytes.

The number of such cases, either of the leukemic or of the aleukemic type, in which systemic lesions of the hematopoietic system could be established is rather limited; moreover, this question is of such theoretical importance that a detailed description of the following case seems justified.

## REPORT OF CASE 1

A man, 75 years old, admitted to the hospital on account of injuries to his right shoulder and hip from falling, died the next day. There was found an intra-trochanteric fracture of the right femur. All the bones of the pelvis and of both shoulders, the skull and the spine showed considerable mottling on roentgen examination. The roots of the lungs were infiltrated.

*Autopsy.*—At autopsy, the spleen was considerably enlarged and firm. The capsule was thick and milky. On section, it was smooth and showed a peculiar pattern, due to irregular, pale, pinkish-yellow and often coalescing areas in the pulp. The trabeculae stood out well. The follicles were hardly visible.

The liver was of medium size and rather hard, the surface smooth and the capsule thin and transparent. On section, a fine trabecular pattern of white color was noted. It spread irregularly over most of the reddish-brown parenchyma.

The abdominal lymph nodes were enlarged, the largest occurring about the portal region. They were soft, and on section were finely granular and white. Other enlarged nodes were found in front of the vertebral column and in the right inguinal region.

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Wedges cut out from the lumbar vertebrae showed the substitution of the spongy bone by a compact yellowish tissue. In between there were scattered reddish areas, containing islands of spongy bone with marrow. The ribs also showed substitution of their spongy structure by compact, sclerotic, yellowish-white tissue.

The neck of the right femur was fractured. The surrounding soft tissues were infiltrated with blood. There was a cavity in the neck of the femur about the size of a goose egg extending upward to the head on one side and downward to the greater trochanter on the other. The adjacent bony tissue was brittle, and between the fragments of the broken wall of the cavity was soft, moist, dark red tissue. Below the cavity, the shaft showed substantial, compact bony cortex with an unusually wide marrow cavity, filled with a rather solid, grayish-red marrow.

*Histologic Examination.*—The tissue infiltrating the bones in the vicinity of the fracture, as well as in the ribs and vertebrae, consisted of spindle-shaped cells with ample cytoplasm and bipolar processes that occasionally seemed to continue into fibrils. The nuclei were elongated or oval and vesicular, and contained fine chromatin granules. Between the spindle-shaped cells there were others, round and containing round or bean-shaped nuclei. There were also numerous myelocytes, many with eosinophilic granules, and mononuclear agranular cells that looked like myeloblasts. Typical bone marrow giant cells and occasional lymphocytes also occurred. Some of the tumor cells had larger, even giant nuclei, but they were always poorer in chromatin than the myeloplakes. Sections stained with Mallory's aniline blue-orange mixture revealed a fine network of pale-bluish fibrils interconnecting the spindle-shaped cells. Silver impregnation showed that these were reticulum fibrils.

In the spleen, very small atrophic follicles and heavy trabeculae were seen. The pulp was rather cellular in places, but presented also extensive areas of fibrosis. Brownish-yellow pigment was present and particularly abundant in the periphery of the fibrotic areas. Under high power, these fibrotic areas showed small, spindle-shaped cells embedded between hyaline fibrils. The cellular part also contained large collections of spindle-shaped cells, numerous polymorphonuclear cells and myelocytes, many of which were eosinophilic. The brown pigment was partly extracellular and partly inside the reticulum cells. Some of the splenic sinuses were obliterated; others were patent and lined by small cuboidal or flat cells. Mallory's stain showed a dense network of fibrils, coarse and dark blue in the fibrosed areas, and fine and pale purplish in the cellular areas. Silver impregnation revealed a dense network of fine argentophil fibers throughout the pulp, with coarse fibrils in the fibrous areas. The small lymph follicles were the only places free from these fibrils.

The lymph nodes presented a plexiform arrangement. The plexuses were separated by loose connective tissue, holding many small blood and lymph vessels. Occasionally, the plexuses showed remnants of lymphatic tissue, particularly those at the periphery of the node. The preponderant cells, however, were the same, mostly spindle-shaped cells, described in the bone marrow. They grew sometimes in the form of parallel fibrillar strands. Silver impregnation showed a rather dense network of argentophil fibrils, in the meshes of which were embedded pale-yellowish reticulum cells. Groups of lymphocytes, where present, stained darker and showed almost no intercellular fibrils. Mallory's stain brought out the same points.

The hepatic cells contained a good deal of hemosiderin, especially those in the periphery of the lobules. The periportal connective tissue was rather cellular. There were also cellular foci without any relation to the periportal fields. Some of the smaller foci were apparently developed in the spaces between two hepatic trabeculae, while the larger nodules seemed to replace the hepatic tissue, as shown

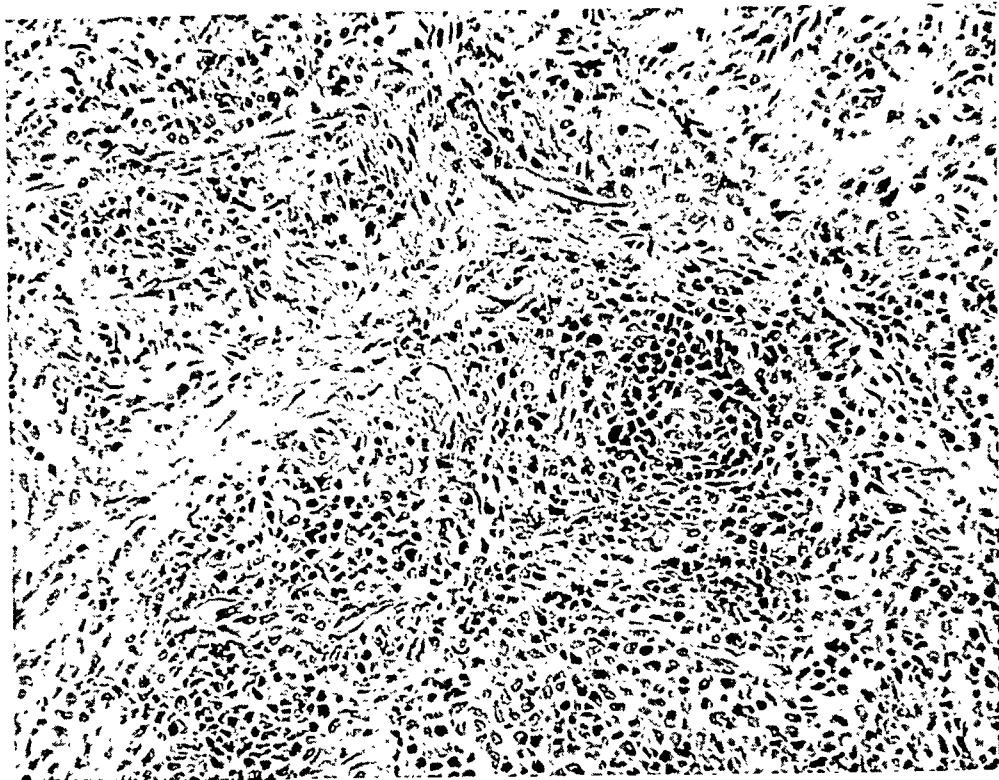


Fig. 1.—Spleen, showing remnant of malpighian follicle surrounded by spindle-shaped reticulum cells, and an adjacent area of fibrosis.

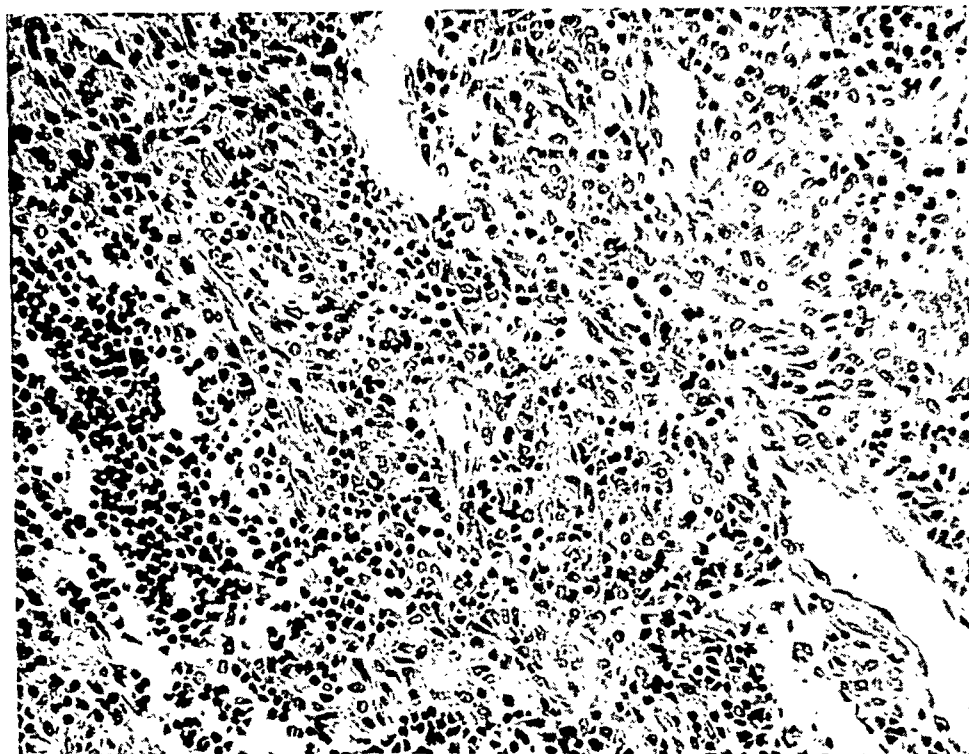


Fig. 2.—Lymph node, showing lymphatic tissue replaced by proliferated reticulum cells, and wide lymphatic sinuses lined with endothelium.

by occasional remnants of hepatic cells within the nodules. Most of the cells were of the spindle-shaped type, but in between there were also lymphocytes, polymorphonuclear cells, myelocytes and numerous eosinophils. Examination under the high power lens showed accumulations of elongated cells in the capillaries even at a distance from the larger nodules. These cells were either identical with, or resembled closely, Kupffer cells. In some capillaries, the Kupffer cells could be seen in the process of division, two or three of these cells taking the place of one. Other capillaries showed larger groups of cells, some of which were also larger in size. Still others were not only plugged by such cells, but also visibly distended by their accumulation until eventually the nodular stage was reached. Thus was seen an unbroken chain from the division of the single Kupffer cell to the formation of the grossly visible nodule.

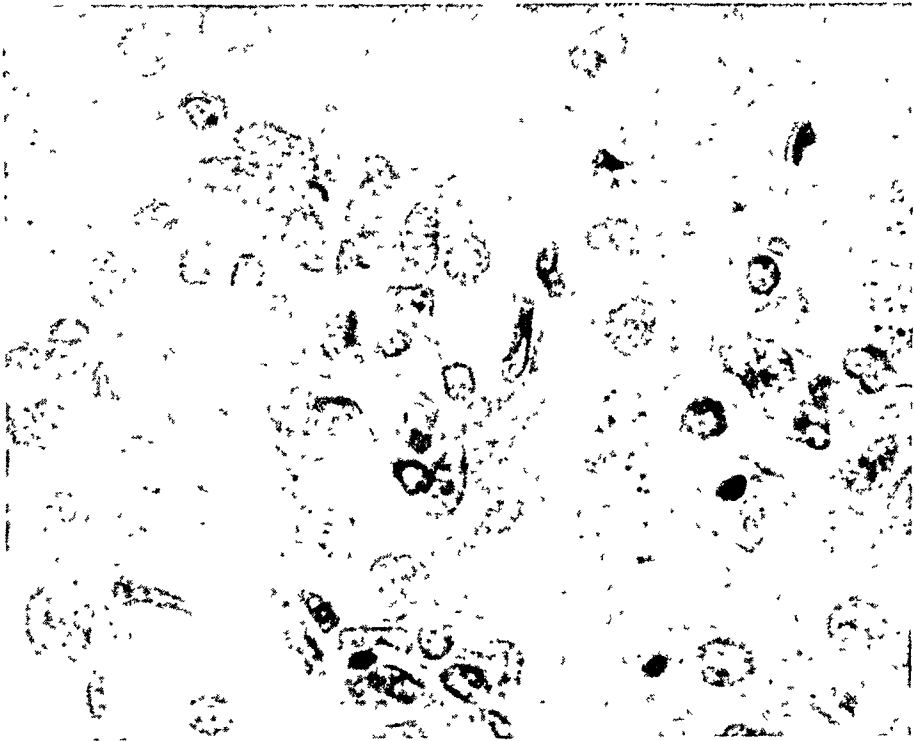


Fig. 3.—Liver, showing nodule formed by proliferation of Kupffer cells.

In searching the literature, we were able to find twenty-two similar cases.<sup>1</sup>

1. Akiba, R.: *Virchows Arch. f. path. Anat.* **260**:262, 1926. Bock, H. E., and Wiede, K.; *ibid.* **276**:553, 1930. Borissowa, A.: *ibid.* **172**:108, 1903. Derischonoff, S. M.: *Frankfurt. Ztschr. f. Path.* **41**:184, 1931. Ewald, O.: *Deutsches Arch. f. klin. Med.* **142**:222, 1923. Feller, A., and Risak, E.: *Folia haemat.* **43**:377, 1931. Goldschmidt, E., and Isaac, S.: *Deutsches Arch. f. klin. Med.* **138**:291, 1922. Goedel, A.: *Frankfurt. Ztschr. f. Path.* **29**:375, 1923. Hittmair, A.: *Folia haemat.* **37**:321, 1928. Letterer, E.: *Frankfurt. Ztschr. f. Path.* **30**:377, 1924. Paine, C. G.: *J. Path. & Bact.* **34**:139, 1931. Pentmann, I.: *Frankfurt. Ztschr. f. Path.* **18**:121, 1916. Reschad, H., and Schilling, V.: *München. med. Wchnschr.* **60**:1981, 1913. Richter, M. M.: *Am. J. Path.* **4**:285, 1928. Roulet, F.: *Virchows*

In addition to these systemic cases, we found a number of others in which only a specimen of tissue from a lymph node or a tonsil was examined.<sup>2</sup> The changes were comparable with those observed in the lymph nodes in some of the systemic cases. One case (Roulet's case 8) came to autopsy. It showed also involvement of the mediastinum, lungs and thyroid gland. Myelogenous leukemia seems to have been present in one other case (Roulet's case 9). We also observed a case of this type in which the clinical picture was suggestive of a systemic lesion.

#### REPORT OF CASE 2

A man, aged 35, complained of pain in the right hip, in both flanks, and in the epigastrium and of great loss of weight. The spleen was enlarged, and there was a chain of palpable glands in the neck. The temperature ranged between 101 and 104 F.; the urine contained casts and leukocytes; there were marked secondary anemia and leukocytosis with 13 per cent eosinophils and occasional myelocytes. Roentgen examination showed destruction of the body of the right os innominatum, internal to the acetabulum. The patient was discharged after one week and shortly afterward died.

Biopsy of a lymph node showed complete obliteration of the original structure. There were still lymphocytes left in the tissue, but they were irregularly scattered throughout. Instead there were irregularly distributed cells of odd shapes and varying sizes. Most of these were large, and their cytoplasm was more or less abundant; yet two different cell types could be distinguished: One assumed a pale stain and had fibrillar, not collagenous, stroma. The nuclei of these cells were vesicular and pale and occasionally showed nucleoli. The other cell type was conspicuous for the intense basophilia of the cytoplasm. It stained so intensely with hematoxylin that it was often impossible to distinguish the nucleus from the cell body. The nuclei were hypochromatic and oddly shaped. Some showed evidence of karyorrhexis; others showed atypical mitotic figures. Transition forms between the two major cell types were common.

The large basophilic cells had a tendency to form groups that were often almost trabecular. Some of them were also found within the lumen of the larger lymphatic spaces, which were lined with a flat endothelium or occasionally with cells that seemed to participate in the changes described.

Another very small lymph node showed almost normal structure with a few secondary follicles, except for an overabundance of lymph spaces which were lined with endothelium, often large and of peculiar appearance. There was also a definite hyperplasia of reticulum cells, many of which had taken up pigmented particles. A few scattered reticulum cells were conspicuous for their irregular hyperchromatic nuclei. Such cells were somewhat more numerous in the center of the secondary follicles.

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Arch. f. path. Anat. **277**:15, 1930. Sachs, F., and Wohlwill, F.: *ibid.* **264**:640, 1927. Schultz, A.; Wermbter, F., and Puhl, H.: *ibid.* **252**:519, 1924. Swirtschewskaja, B.: *ibid.* **267**:426, 1928. Terplan, K.: *Verhandl. d. deutsch. path. Gesellsch.* **25**:69, 1930. Terplan, K., and Mittelbach, M.: *Virchows Arch. f. path. Anat.* **271**:259, 1929. Tschistowitsch, T., and Bykowa, O.: *ibid.* **267**:91, 1928. Ugriumow, B.: *Centralbl. f. allg. Path. u. path. Anat.* **45**:103, 1928.

2. Komocki, W.: *Virchows Arch. f. path. Anat.* **250**:517, 1924; **277**:605, 1930. Roulet, F.: *ibid.* **277**:15, 1930.

## COMMENT

In reviewing the previously reported cases of similar character, we were impressed with one feature which was common in all of them as well as in our own: the proliferation of the reticulum cells in a manner that it is fair to call systemic. The most generalized condition was that described by Bock and Wiede, who observed a case of monocytic leukemia with infiltration of the liver, spleen and lymph nodes. The changes in the bone marrow were rather insignificant, which is the more remarkable as the proliferation of histiocytes throughout the connective tissue seemed to indicate a rather generalized activation of the mesenchyma.

The most frequently involved organ was the spleen, with the liver second. The bones and the bone marrow also showed changes in most of the cases. The lymph nodes, which were involved in the majority of the cases, were the main seat of the lesion in some of Roulet's series and in Goedel's case. It remains questionable whether the involvement was really systemic in our second case, in those of Komocki and in several others reported by Roulet. Only in some cases did the proliferation of the reticulum cells assume such proportions as to suggest a malignant neoplasm. These cases include our own second case, Schultz and associates', Richter's and three of Roulet's series.

It seems beyond reasonable doubt that the proliferating cells came from the so-called reticulum cells of the spleen, bone marrow and lymphatic tissue and from similar cells in the liver. The variety of appearance of these cells was noticed in most of the cases and brought out strikingly in ours. The cells were often spindle-shaped and showed a tendency to participate in the production of intercellular fibrils. The nature of these fibrils was not studied carefully in most of the cases. In our case, as shown by van Gieson's and Mallory's stains and by silver impregnation, the fibrils appeared to belong to the reticulum and not to the collagenous fibers. Goedel reported similar observations.

However, the spleen in our case showed areas of definite fibrosis, that is, the development of collagenous fibrils, which stained a bright red with van Gieson's stain and did not impregnate with silver. Borisowa described similar fibrotic areas in the spleen in her case. In view of the fact that gradual transition could be observed from the argentaftine reticulum to the areas of collagenous fibrosis, we are of the opinion that the fibrotic areas represented a late stage in the lesion. This would indicate that the pathologic reticulum cells may acquire fibroblastic properties in the sense that they produce collagenous connective tissue. A fibrotic replacement of structures previously built by reticulum cell proliferation is conceivable; yet our sections did not show any proliferation of fibroblasts, nor any such invasion of the

areas in question. The burden of the proof that reticulum cells do not form collagenous tissue falls on those who are not willing to admit this possibility.

The proliferated reticulum cells were also described by many authors as polygonal with scanty cytoplasm, yet showing processes that connected with the fibrillar network of the reticulum. Pentmann and others described the occurrence of giant cells in the lesions. We saw such cells in the bone marrow. Most of the authors believed that these giant cells were the result of incomplete amitotic division. They varied in size, were irregular, and contained as many as six nuclei with a fine chromatin network and distinct spherical nucleoli. The cell body showed indistinct outlines and coarse processes. The proliferation of these cells at times led to the formation of multinucleated protoplasmic masses of peculiar appearance.

Another type of cell that occurred in our case as well as in those of Akiba and Roulet was a large, round cell with round nucleus, which did not show fibrillar connection with the reticulum, and which was found free either in the meshes of the reticulum or in the open lumen of lymphatic and blood capillaries. This cell was, as a rule, distinctly basophilic. Its cytoplasm was in many cases more abundant than that of a myeloblast. Transitions between this round cell type and the spindle-shaped form were clearly demonstrable. It appeared to be a cell that had come from the same matrix, but that had lost its ability to produce reticulum fibrils. In other words, the cell was less differentiated and was reverting to the type of a more primitive mesenchymal cell.

The intertrabecular blood spaces in the liver showed extensive accumulations of cells, not only in the cases of leukemic type, but also in those in which the blood did not disclose evidence of leukemia. Moreover, proliferation of spindle-shaped cells was clearly demonstrable. Their morphology excluded the possibility that we were dealing with hematogenous cells. The question arises whether these cells were products of the lining endothelium of those sinuses, or whether they derived from other mesenchymal cells located in the intertrabecular spaces and not forming part of the endothelial lining. It hardly seems justifiable to distinguish between phagocytic mesenchymal (Kupffer) cells and lining endothelium. It is highly probable that the phagocytic stellate cell represents only a functional stage in the life cycle of the intertrabecular hepatic capillary endothelium.<sup>3</sup> We believe, therefore,

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3. Goldzieher, M. A., and Peck, S. M.: Experimental Studies on the Reticulo-Endothelial System: I. Response to Infection, *Arch. Path.* **3**:629, 1927; II. Effect of Mercury Salts and Sulpharsphenamine on Reticulo-Endothelial Cells, *ibid.* **3**:635, 1927.

that the lining endothelium of the hepatic capillaries participates in the systemic proliferation of the reticulum cells.

Of special interest is the proliferation of the endothelium of the splenic sinuses, which was noticeable in several cases. The case of Pentmann is particularly noteworthy because the changes were accompanied by the formation of a large, cavernous, angiomatic nodule in the spleen and also in one of the vertebrae. These observations would indicate that the sinus endothelium in the spleen and liver is not as remote from the reticulum cells as it was claimed at one time to be. They represent, indeed, an instance of special differentiation that serves different purposes, and this is expressed by morphologic dissimilarity. Essentially, however, these cells are all derived from the same primitive mesenchymal cell. Although they represent products of diverging differentiation, still they belong to the same class of reticulo-endothelial cells and may participate in the changes which befall, as a rule, more conspicuously the reticulum cells proper.

The real nature of the lesion described is difficult to define. This is brought out by the diversity in the nomenclature. Some of the cases were described as granulomatous lesions and others as leukemia. Some authors chose the noncommittal term of hyperplasia of the reticulo-endothelial apparatus, while others selected the new terms of reticulosis, reticuloma and reticulo-endotheliosis. In some of the cases, the neoplastic character of the lesion was so striking that the term reticulosarcoma or retothelsarcoma was applied. This list of more or less suggestive names should not include the misleading terms used by some authors, such as angiosarcoma.

In our first case, the lesion of the bones was certainly suggestive of a malignant neoplasm, whereas the appearance of the spleen, lymph nodes and liver indicated a systemic involvement of hyperplastic rather than of neoplastic nature. It is well known how difficult it is to draw sharp lines of demarcation between cases of systemic involvement of the hematopoietic apparatus. We refer to the leukosarcoma of Sternberg, in which leukemia is associated with a growth of unquestionably sarcomatous behavior and microscopic appearance. We also refer to Hodgkin's disease, in which the systemic formation of granulation tissue is not infrequently associated with aggressive, sarcoma-like proliferation of the newly formed tissue. These cases were spoken of by Ewing as Hodgkin's sarcoma.

Roulet drew attention to the similarity between certain conditions described as lymphosarcoma and what he calls retothelsarcoma of the lymph nodes. It is obvious that many of the older authors, including Ghon and Roman,<sup>4</sup> described sarcoma of reticulum cells as lympho-

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4. Ghon, A., and Roman, B.: *Frankfurt. Ztschr. f. Path.* **19**:1, 1916.

sarcoma. Most of these were not of a systemic type, while others approached a degree of dissemination that may bring them within the fold of systemic lesions. It is important to stress that none of the cases, with the exception of Pentmann's, showed any attempt at differentiation into structures of endothelial character. This applies also to Paine's case, although he mentioned a tubular structure, but one without definite lumen. His choice of name, "angiosarcoma," does not seem to be warranted by his own description. None of the lesions of the bone marrow cited in this paper showed any of the features characteristic of endothelioma of bone. The latter, and particularly Ewing's diffuse endothelioma, seem to come from cells that maintain a higher degree of differentiation, remaining specialized endothelial cells, notwithstanding their neoplastic tendencies and their derivation from the same matrix that is involved in the systemic process investigated in this paper. Careful comparison with several cases of Ewing's tumor has convinced us that it is impossible to differentiate histologically the tumor-like lesions in cases of systemic involvement of the reticulo-endothelium from Ewing's diffuse endothelioma of bone.

There are no definite criteria at present by which one can classify a lesion as neoplastic instead of hyperplastic. Thus it seems justified to stress the systemic nature of the lesion described and to designate it with the somewhat indefinite term of reticulosis, in order to distinguish it from related, but more localized and apparently more definitely aggressive lesions (reticulum sarcoma).

The relationship of the term reticulosis, in the sense applied by us, to the more commonly used terms, myelosis and lymphadenosis, may cause some misunderstanding. Both these terms are used instead of the ancient expression leukemia to designate a systemic disease in which myeloid or lymphatic cells are formed in excess, and in which many of these cells do not reach a stage of full differentiation and maturity. Both myelosis and lymphadenosis may or may not be accompanied by invasion of the blood stream, that is, by the clinical symptoms of leukemia. Essentially, however, both forms—whether leukemic or aleukemic—consist of a systemic hyperplasia of myeloid or lymphatic cells, respectively. Reticulosis, in contradistinction to both, represents hyperplasia of the third main constituent of the hematopoietic tissues.

It stands to reason that reticulosis may also occur in a leukemic form. The dominating cell type in these cases is the monocyte, and the literature on monocytic leukemia includes already a number of reports of well studied cases. The reticulo-endothelial origin of these cells was demonstrated in several of these cases by numerous transition forms in the circulating blood between mature monocytes and their stem cells, and particularly by the evidence that many of the proliferating reticulo-endothelial cells were being mobilized, since they were



found free in the lumen of hepatic or splenic sinuses. Richter described his case as one of lymphatic leukemia and Roulet his as one of myelogenous leukemia, yet without any details as to the blood picture or as to changes in the bone marrow. Thus it is impossible to decide whether these cases represent merely a coincidence of leukemia with reticulosis, or whether the leukemic blood changes were in some way based on proliferation of reticulum cells.

#### SUMMARY

A case of generalized proliferation of the reticulo-endothelial cells, with involvement of the bones, the spleen, the liver and lymph nodes, is described. Another case is described in which a comparable lesion developed from the reticulum cells of the lymph nodes. The relationship of both cases to others previously reported is discussed. The name reticulosis is suggested for this process, which may or may not associate itself with leukemia. Reticulosis cannot be always sharply demarcated from neoplastic conditions, and a number of cases remain on the borderline between reticulosis and reticulum cell sarcoma. Instances of the latter, which localizes in one place (lymph node or tonsil) have been previously classified as lymphosarcoma, Hodgkin's sarcoma, or endothelioma of lymph nodes.

# THE GRADING OF CARCINOMA OF THE CERVIX UTERI AS CHECKED AT AUTOPSY\*

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Although numerous attempts have been made to evaluate the clinical significance of histologic variations in malignant tumors,<sup>1</sup> the subject is still far from settled.<sup>2</sup> Various factors have been introduced in an effort to increase the accuracy of histologic grading. Some of these factors are anatomic, such as the location and the extent of the tumor, but clinical factors have been introduced as well, in an effort to maintain correlation between the grading and the end-result. Even economic status deserves consideration.

The inherent difficulties in any attempt to use duration of life as a criterion for the efficacy of the histologic grading of tumors are obvious. Thus, when any such scale is used, if it is to be at all accurate, it must allow for the normal duration of life in the untreated patient, the adequacy or inadequacy of treatment in the individual case, the fact that occasionally a patient dies as a result of treatment rather than of the disease itself and finally the fact that death may occur from entirely unrelated causes. Moreover, many of the patients do not die from carcinoma as such, but from related mechanical injuries, such as hydro-nephrosis.

A renewed incentive to attempts at grading was given by Ewing,<sup>3</sup> who pointed out that the histologic appearance often affords important clues to the radiosensitivity or radioresistance of a given growth.

Since most of the studies have used clinical criteria, particularly the duration of life after the onset of the disease, for the determining of the degree of malignancy, it has seemed worth while to attempt a study using largely pathologic data.

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\* From the Pathological Laboratories of the New England Deaconess and the Collis P. Huntington Memorial Hospitals, and the Department of Pathology, Harvard Medical School.

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2. Plaut, A.: Relation of Prognosis to Histologic Findings in Carcinoma of Cervix, *Surg., Gynec. & Obst.* **43**:450, 1926. Reimann, S. P.: Issues at Stake in the Grading of Tumors, *Arch. Path.* **8**:803, 1929.

3. Ewing, J.: Radiosensitivity, *Radiology* **13**:313, 1929.

The present study is based on the observations at autopsy in one hundred and two cases of carcinoma of the cervix uteri. The power of a tumor to metastasize has been taken arbitrarily as an index of its degree of malignancy. While perhaps the power of metastasis is a one-sided criterion of malignancy, its use has certain definite advantages. The personal equation is largely eliminated. Variations in type and efficiency of treatment are compensated for to some extent. Variations in clinical factors may be largely neglected. Intercurrent disease processes have little influence.

The primary tumors were graded; the sections were compared with the previous biopsy specimens, if any, and then the distribution of metastases was studied. A modification of Broder's classification was used, dividing the epidermoid carcinomas, on the basis of differentiation of the tumor cells, frequency of mitosis and relation of tumor to stroma, into three groups: group 1, of low malignancy; group 2, of medium.

TABLE 1.—*Distribution of Cases by Types*

Type and Grade	Cases	Average Age of Patient at Death	Average Duration of Disease*
Epidermoid Carcinoma:			
Grade 1.....	23	53 yr., 7 mo.	2 yr., 9 mo.
Grade 2.....	44	51 yr.	2 yr., 1 mo.
Grade 3.....	18	45 yr., 4 mo.	1 yr., 2 mo.
Adeno-acanthoma.....	6	53 yr., 3 mo.	2 yr., 3 mo.
Adenocarcinoma.....	8	57 yr., 6 mo.	3 yr., 6 mo.
Cases in which all tumor was destroyed.....	3	47 yr., 7 mo.	1 yr.
Total.....	102	51 yr., 5 mo.	2 yr., 2 mo.

\* Duration unknown, 2 cases.

and group 3, of high. In addition, the adeno-acanthomas (combined epidermoid and adenocarcinoma) and the adenocarcinomas of the cervix have been included.

In three cases, all of the tumor had been destroyed by the treatment and no metastases were found, death resulting in one of the cases from cachexia and in the other two from sepsis. These cases are particularly worthy of note, in that the short duration of life after treatment, averaging 7.6 months, would be considered as evidence of the presence of a highly malignant type of tumor.

In table 1, the distribution of cases by grade and type is shown, with the average age of the patients at death. As can be seen in table 1, a considerable proportion of the cases fall into grade 2, those of moderate malignancy. This is not because grade 2 is merely a scrap-bag for cases that do not fall definitely into group 1 or group 3, as only the cases are included in each group that meet the criteria for that group.

The average duration of all cases of the disease, both those in which there was treatment and those in which there was not, was 2.2 years.

In table 2 the types of treatment are given. It will be seen that the duration from the apparent onset of the disease to treatment is long in all cases, except the highly malignant cases of grade 3, the progress of which forced the patients to seek treatment within 7 months, on the average. In striking contrast are the three cases in which all the tumor was destroyed by treatment; in these the duration of symptoms before treatment had been only 4.7 months.

The biopsy specimens of the tumors that were entirely destroyed by treatment showed them to have been epidermoid carcinomas, grade 2.

TABLE 2.—*Types of Treatment*

	Cases	Epidermoid Carcinoma			Adeno- acan- thoma	Adeno- carci- noma	Cases in Which All Tumor Was Destroyed
		Grade 1	Grade 2	Grade 3			
Total number of cases.....	102	23	44	18	6	8	3
Untreated.....	19	6	10	2	1	..	..
Treated by operation only.....	6	..	4	1	..	1	..
Treated by radium only.....	46	12	16	11	2	4	1
Treated by x-ray only.....	1	..	1	..	..	..	..
Treated by operation and radium.....	14	1	8	2	1	1	1
Treated by operation and x-ray.....	2	..	..	..	1	1	..
Treated by operation, radium and x-ray..	3	1	1	1	..	..	..
Treated by radium and x-ray.....	11	3	4	1	1	1	1
Average duration until treatment*.....	11 mo.	1 yr., 1 mo.	11 mo.	7 mo.	11 mo.	1 yr., 9 mo.	5 mo.

\* Duration unknown, 6 cases.

TABLE 3.—*Distribution of Metastases*

Type and Grade	Cases	Metastases Absent	Metastases to Regional Lymph Nodes		Metastases to Distant Lymph Nodes		Visceral Metastases	
			Lymph Nodes	Lymph Nodes	Lymph Nodes	Lymph Nodes	Cases	Percentage
Epidermoid Carcinoma:								
Grade 1.....	23	13	7	3			1	4
Grade 2.....	44	10	33	20			16	34
Grade 3.....	18	2	14	14			12	67
Adeno-acanthoma.....	6	..	5	3			4	67
Adenocarcinoma.....	8	3	5	3			2	25

This is in accord with clinical studies, which have shown grade 2 carcinomas of the cervix to respond best to irradiation.

Of minor interest is the frequent occurrence of vesicovaginal and rectovaginal fistulas in these cases. While of the forty-five patients in whom fistulas developed, thirty-two were radium-treated, there is no significant difference in the incidence of fistulas in the radium-treated patients and in the untreated patients or those treated with x-rays or operation, fistulas developing in 43 per cent of the radium-treated ones and 46 per cent of the others. In a few radium-treated patients, however, the character of the tissue surrounding the fistulas strongly suggested radiation necrosis as the cause for their presence.

The distribution of metastases is presented in table 3.

Of the epidermoid carcinomas, grade 1, thirteen, or 57 per cent, had no metastases, and only one, or 4 per cent, had visceral metastases. Of those of grade 2, only 23 per cent were without metastases; 77 per cent showed metastases to lymph nodes, and 34 per cent showed visceral metastases. Metastases were the rule with those of grade 3, only 11 per cent remaining localized, whereas 67 per cent had visceral metastases. Not one of the adeno-acanthomas remained localized, and again 67 per cent showed visceral metastases. The adenocarcinomas of the cervix are definitely less malignant than the adeno-acanthomas, 38 per cent remaining localized and only 25 per cent showing visceral metastases.

From these figures it may be seen that there is a close correlation between the histologic degree of malignancy of a tumor of the cervix uteri and its power to set up metastases. One epidermoid carcinoma of grade 1 and two of grade 2 developed in the retained cervix.

Six instances of multiple malignancy were encountered in the series: One was a very early adenocarcinoma of the fundus uteri, co-existing with an epidermoid carcinoma, grade 1, of the cervix; another was a carcinoma of the stomach, co-existing with an epidermoid carcinoma, grade 1, of the cervix; three were malignant adenomas of the rectum, two co-existing with an epidermoid carcinoma, grade 1, and one with an epidermoid carcinoma, grade 2, and there was one case of renal adenocarcinoma of the kidney co-existing with an epidermoid carcinoma, grade 3.

By far the commonest cause of death in the group was uremia resulting from ureteral obstruction, renal infection or a combination of the two. This caused one third of the deaths. Other important causes were cachexia, peritonitis, pneumonia, hemorrhage and intestinal obstruction.

#### SUMMARY AND CONCLUSIONS

A comparison of the distribution of metastases in one hundred and two cases of carcinoma of the cervix uteri in which autopsies were performed shows close correlation between the degree of malignancy as estimated by histologic grading and the distribution of metastases.

Metastasis is uncommon in epidermoid carcinoma, grade 1, and visceral involvement is very rare. In epidermoid carcinoma, grade 3, and in the adeno-acanthomas, metastasis to lymph nodes is the rule, and visceral metastases are common. So far as distribution of metastases is concerned, the degree of malignancy of epidermoid carcinoma, grade 2, and of adenocarcinoma of the cervix is approximately the same.

Impairment of renal function is the most common cause of death in cases of carcinoma of the cervix uteri.

# EXPERIMENTAL PATHOLOGY OF THE LIVER

STUDIES III, IV AND V\*

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## III. THE EFFECT OF DIVERTING THE PORTAL BLOOD ON THE RESTORATION OF THE LIVER AFTER PARTIAL REMOVAL

One of the most outstanding characteristics of the liver is its restoration following injury or partial removal. Fishback<sup>1</sup> reviewed the previous work concerning this characteristic and presented our data with regard to it, which were collected from the observation of dogs, and Higgins and Anderson<sup>2</sup> reported the results of experiments on rats. The capacity of the liver to be restored after partial removal has been the main difficulty in producing a decrease of hepatic tissue and a decrease of function. It seemed of both clinical and experimental value to attempt to determine the cause or causes for such reaction, and whether any experimental conditions would impair the restorative ability of the liver after injury or loss of a portion. If this could be done, a method might be established for permanently reducing hepatic tissue and for studying reduced hepatic function. It might be of aid clinically in determining some of the factors on which such restoration occurs, and furnish a basis for the causes of the varied and characteristic changes found in cirrhosis. Several years ago, Mann and Magath<sup>3</sup> noted that restoration of the liver did not occur in an animal with Eck fistula. We shall present here more complete data concerning partial removal of the liver following diversion of the portal blood from that organ.

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\* Submitted for publication, July 21, 1931.

1. Fishback, F. C.: A Morphologic Study of Regeneration of the Liver After Partial Removal, *Arch. Path.* **7**:955, 1929.

2. Higgins, G. M., and Anderson, R. M.: Experimental Pathology of the Liver: I. Restoration of the Liver of the White Rat Following Partial Surgical Removal, *Arch. Path.* **12**:186, 1931.

3. Mann, F. C., and Magath, T. B.: The Production of Chronic Liver Insufficiency, *Am. J. Physiol.* **59**:485, 1922.

*Methods.*—The experiments were carried out on dogs. All operative procedures were done under ether anesthesia, and with the employment of sterile technic. The portal vein was anastomosed to the vena cava by the modified cutting suture of Fischler and Schröder<sup>4</sup> as developed in our laboratory and as described by Fishback. After the Eck fistula had been made, the animals were carefully observed. These animals do not maintain nutrition well unless special care is exercised in their diet; it has been found that they do best on a diet of milk and syrup without meat or other concentrated protein. From 2 to 3 months after the portal blood had been diverted, two lobes of liver were removed. The technic of this procedure has been described by Fishback. A minimum of 30 per cent of the organ was removed. It was found that the intravenous administration of dextrose for several days following operation was a distinct benefit. The same care with regard to diet was necessary after the liver had been partially removed as after the Eck fistula had been made. Even with the utmost care some animals did not do well, and frequently 3 or 4 weeks after the second operation a duodenal ulcer developed and perforated. In some experiments, exploration was carried out at frequent intervals; the liver was examined for evidence of restoration and a specimen was removed for histologic examination. In other experiments, the animals were killed at stated intervals after the second operation, and the condition of the liver was noted.

*Results.*—Our observations of the effect of Eck fistula on the liver are similar to those described by Whipple and Hooper.<sup>5</sup> From six to ten weeks after diversion of the flow of portal blood, the liver assumed a characteristic condition. On gross examination, the most striking changes were in size and color. The liver of an animal with Eck fistula atrophied to about half its normal size. Its shape and contour remained unchanged, but there was a decrease in all dimensions to about half the normal, and it became characteristically pale, mottled and yellow. Microscopic examination revealed atrophy of the central portions of all lobules, with marked accumulation of fat in the hepatic cells of these regions. Frequently the accumulation of fat was so great that only a rim of the protoplasm of the hepatic cell with a nucleus pushed to the periphery of the cell remained. Although changes occurred first in the central portion of the lobule, they were not always confined to this region, but extended also by ramification to the periportal region. After the atrophy had reached a certain point, it appeared to remain stationary.

After the liver had been partially removed from the animal with Eck fistula, the remaining portion usually remained unchanged. This is in marked contrast with the remaining portion of the liver after partial removal from a normal dog, in which the edges of the remaining lobes become rounded and the lobes dome-shaped, and the total mass of hepatic tissue becomes approximately the same as before operation.

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4. Fischler, F., and Schröder, R., quoted by Fishback, F. C.: Anastomosis of Portal Vein with Inferior Vena Cava; a Thoroughly Tested and Satisfactory Method of Making an Eck Fistula, *Ann. Surg.* **86**:436, 1927.

5. Whipple, G. H., and Hooper, C. W.: Bile Pigment Metabolism: VI. Bile Pigment Output Influenced by the Eck Fistula, *Am. J. Physiol.* **42**:544, 1917.

When the portal blood was diverted, the edges of the remaining lobes remained sharp, and the organ as a whole maintained its atrophic condition. In rare instances in which increase in size was noted, blood vessels carrying adhesions were always present. Microscopically, the liver of the animal with Eck fistula after partial removal differed little from its appearance before partial removal. In some experiments it seemed that there were fewer cells that appeared normal and more cells that contained fat.

*Summary.*—The portal blood was diverted from the canine liver by an Eck fistula, and after the liver had assumed the characteristics of Eck fistula, the effect of removing various amounts of the organ on the remaining portion was observed. Whereas partial removal of the normal liver is always followed by restoration of hepatic tissue, such regeneration did not occur when the portal blood was diverted.

#### IV. THE EFFECT OF PREVIOUS LIGATION OF THE COMMON BILE DUCT ON RESTORATION OF THE LIVER FOLLOWING ITS PARTIAL REMOVAL IN DOGS

There are many methods of injuring the liver; probably the most common clinical method is obstruction to the outflow of bile. The extent to which obstruction of the common bile duct affects the fundamental activities of the liver either transitorily or permanently has not been clearly indicated. The purpose in this research was to determine the effect of obstruction of the outflow of bile on one of the most outstanding reactions of normal hepatic tissue, that is, restoration after partial removal.

*Methods.*—All operations were performed on dogs under ether anesthesia, with rigid aseptic technic. The common bile duct was exposed, doubly clamped, cut and doubly ligated with linen about 1 cm. from its entrance into the duodenum; the duodenal end was not ligated. The gallbladder was removed, because it had been found that perforations of the cystic and common bile ducts are more common when the gallbladder becomes distended after obstruction to the outflow of bile. In order to have a specimen of normal liver for comparison, a triangular wedge of tissue was removed from the border of the right ventral lobe and placed immediately in Zenker's solution; the wound was closed with mattress sutures of catgut. The weight of the liver was estimated at this time from its size, from the size and weight of the dog, and from the table of weights of the livers of normal dogs, as outlined by Fishback. After operation the animal was placed on a diet of equal parts of milk and syrup, which had been found to be particularly valuable for the maintenance of animals with obstruction of the common bile duct. At a second operation, from 7 to 52 days after the obstruction of the outflow of bile, the liver was partially removed according to the technic described by Fishback. The resected lobes were weighed, and a specimen was fixed. The weight of the remaining portion was estimated. A minimum of 30 per cent of the organ was always removed, but usually the amount was greater. The appearance of the liver, the degree of dilatation of the common and hepatic bile ducts and the degree



of jaundice of the sclera and viscera were noted. The animal was again placed on a diet of milk and syrup until the termination of the experiment. Animals that did not die when under observation were killed by ether. Necropsy was always performed promptly. The abdominal and thoracic viscera were weighed separately to determine any increase in size. Ligation of the duct was verified, and the duodenum was opened to exclude the possibility of reestablishing drainage of bile. Tissue from the liver was fixed immediately in Zenker's solution. Sections were prepared and stained by the usual technic.

*Results.*—The length of life following ligation of the common bile duct varied greatly. Spontaneous death was usually due to rupture of a duodenal ulcer or to distention of the common bile duct, with peritonitis resulting. Jaundice, perceptible in the sclera from fourteen to sixteen hours after operation, increased rapidly, and reached its maximum within the first ten days. During this period the sclera, skin and mucous membranes became markedly yellow, and the viscera and other tissues, including the cartilage, yellowish to yellowish green. The intensity of the jaundice usually decreased in a few weeks, but normal color of the tissues never returned completely. In a few experiments a large amount of clear fluid was found in the abdomen from five to ten days after ligation of the common bile duct, but in general ascites was usual, although not marked.

At the second operation, the appearance of the liver was typical of that associated with obstruction to the flow of bile. It varied in color from greenish to brownish black and appeared distended and engorged; the individual lobules were prominent. The external surface was smooth and glistening, and frequently some adhesions were present between the duodenum and the under surface of the right central lobe. The common and hepatic bile ducts were usually dilated to about from four to six times their normal caliber, and their walls were thin and friable. The bile in the ducts was usually thin, watery and greenish brown. The microscopic picture consisted essentially of a slight increase in connective tissue in the interlobular spaces directly around the dilated bile ducts; this increase did not extend around the radicals of the portal vein or of the hepatic artery or into the lobule itself. The intrahepatic ducts were usually dilated to about twice the usual size, but the cells lining them appeared normal. In the interlobular spaces and throughout the parenchyma were numerous large and small areas of new bile ducts, which seemed to form whorls of new tissue throughout the organ; around these, connective tissue was slightly increased. The cells of the parenchyma disclosed little, if any, atrophy or other change, but those bordering the areas of new bile ducts were usually more deeply stained. Pigment was scattered irregularly throughout the organ, but apparently in the canaliculi rather than in the cells. Except for the presence of the pigment, the slight increase in connective tissue around the ducts and the areas of

new bile ducts, the organ appeared to be practically normal. There was no evidence of the formation of new cells before partial hepatectomy was performed.

The liver was partially removed from 5 to 52 days after ligation of the common bile duct; the average interval between the two operations was about 30 days. The immediate clinical effect after partial removal was slight. Several of the animals gained weight, were only slightly jaundiced and seemed to be in excellent health at the end of from 131 to 141 days after ligation of the common bile duct, or at the end of from 101 to 111 days after partial hepatectomy. Ascites was not marked when the prescribed regimen was maintained.

Several months after partial resection of the liver there appeared to be less engorgement than after ligation of the duct alone, and the color was possibly brownish red rather than greenish black; however, the organ did not differ strikingly from that in the earlier stages after simple ligation. The obstructed, uninfected organ had not increased in size or weight from 3 to 110 days after partial hepatectomy; this is in strong contrast with the almost complete regeneration of the unobstructed liver that has been demonstrated so often. In the fifth month, or more than 100 days after partial hepatectomy, the caliber of the ducts was about half what it was in the second month, and the walls were thick rather than thin and transparent, as in the earlier stages of distention. The character of the bile in the ducts was similar to that found earlier; namely, thin, watery and greenish brown, although occasionally white. Microscopically, in most cases, from 4 to 30 days after partial hepatectomy, the sinusoids were dilated and the cells of the parenchyma appeared somewhat swollen, but essentially normal. The picture was otherwise that of the liver at about the same interval following ligation of the common bile duct. In other experiments there was moderate proliferation of the cells of the parenchyma, with mitotic figures in various stages, but few in number. In the periphery of the lobule and especially around the whorls of new bile ducts, there were many areas composed of cells that appeared to be newly formed. The typical new cell was smaller than the normal parenchymal cell, and the protoplasm was stained a dark pink with hematoxylin and eosin. Its nucleus was elliptical or oval, very darkly stained and smaller than normal; a nucleolus was not seen. Later there was a more distinct increase in the connective tissue around the bile ducts, with a decrease in the pigment scattered throughout the organ.

Infection played an important part in the jaundiced dogs, and when it developed after either ligation of the common bile duct or partial hepatectomy, the animal did not live for more than a few weeks. When the infection occurred following ligation of the duct, the formation of connective tissue was much more marked than without it; when the infection was of a severe grade, atrophy of the parenchymal cells ensued

rapidly. Infection following partial hepatectomy produced the same changes in the remainder of the liver, but it is worthy of note that mitotic figures and new parenchymal cells were found in some livers in which abscesses were present, although the mitotic figures were not usually found in the lobes that contained the abscesses, and a few of the infected remaining lobes of liver showed a definite increase in size.

*Comment.*—The remarkable restoration of the normal liver after partial removal is in marked contrast with the effect of partial removal after obstruction of the common bile duct. Little, if any, increase in the size or weight of the liver was noted after the operation was performed at varying intervals following ligation of the common bile duct. Shortly after removal of a portion of a liver with obstructed biliary outflow, the hepatic sinusoids became engorged, and occasionally mitotic figures of the parenchymal cells were observed. These changes apparently signified some slight effort toward restoration, but the size or weight did not increase, or increased only to a slight extent; the edges of the liver remained sharp and did not become rounded as in the normal liver after partial removal. It is quite evident that restoration of the liver after partial removal does not occur or occurs only to a slight extent when outflow of bile is prevented.

*Summary.*—A series of experiments was performed on dogs to determine if the liver would be restored after partial removal if the outflow of bile had been previously obstructed. The routine procedures were as follows: (1) ligation of the common bile duct and removal of the gallbladder and (2) removal of two or more lobes of the liver at various intervals after the production of biliary obstruction. It was found that partial removal of the liver was not followed by the remarkable restoration that occurs in the normal liver.

#### V. THE EFFECT OF CIRRHOSIS ON RESTORATION OF THE LIVER AFTER PARTIAL REMOVAL

Previous observations of the restoration of the liver after partial removal have demonstrated that: (1) Removal of a portion of the normal liver is always followed by restoration; (2) if the portal blood has been diverted by an Eck fistula, restoration after partial removal does not occur, and (3) obstruction of the biliary outflow likewise prevents restoration of hepatic tissue after partial removal. Our purpose in this investigation was to determine if restoration of the cirrhotic liver occurs after partial removal.

*Methods.*—Dogs were used in all experiments. Cirrhosis was produced by the administration of carbon tetrachloride by stomach tube; this was done originally by Lamson and Wing.<sup>6</sup> The amount of the drug given varied, depending on its

6. Lamson, P. D., and Wing, Raymond: Early Cirrhosis of the Liver, Produced in Dogs by Carbon Tetrachloride, *J. Pharmacol. & Exper. Therap.* **29**: 191, 1926.

acute effect. The usual amount was from 3 to 5 cc. every 2 to 5 days. From 4 to 8 months and in most experiments 6 months after beginning the administration of the carbon tetrachloride, the animal was etherized, and with aseptic technic the liver was explored, the degree of cirrhosis was noted, and various lobes of the organ were removed. After permitting sufficient time for the restoration of the remaining portion of the liver as it occurs in the normal dog, either exploration was again carried out and the condition of the liver noted, or the dog was killed by bleeding under ether, the liver was examined and weighed, and sections were removed for microscopic examination.

*Results.*—The rate and degree with which cirrhosis developed following administration of the drug varied considerably in the different animals, but cirrhosis was found in all of them. When 30 per cent or more of the liver was removed with only a moderate degree of cirrhosis present, some restoration occurred in the remaining portion, as was evidenced by the rounded edges and dome shape of the lobes, as well as by the increase in weight of the entire organ. However, when cirrhosis was marked, the surface of the liver nodular, and the whole organ atrophic in association with a great increase in connective tissue, little, if any, restoration of the remaining portion occurred after partial removal. Except when cirrhosis was marked, however, slight restoration always occurred. There was more evidence of restoration in the cirrhotic liver than occurred following either diversion of the portal blood or obstruction of the outflow of bile.

*Summary.*—Cirrhosis of the liver was produced in dogs by the administration of carbon tetrachloride by stomach. When cirrhosis was well developed, the liver was partially removed. There was little restoration of the cirrhotic liver after its partial removal as compared with that of the normal liver. Restoration did not occur when the cirrhotic condition was well developed.

# EFFECT OF HOMOLOGOUS MACERATED SKIN ON THE REGENERATION OF EPIDERMIS \*

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Experiments were undertaken to determine the effect of macerated dead tissue on the regeneration of living cells of like kind maintained in contact with their normal blood supply. Many substances varying in character from complex tissue derivatives to simple inorganic salts have been applied to tissues proliferating *in vitro*. In the tissue cultures, in which the conditions of food supply are relatively simple, the mere addition or withholding of a substance from the medium may be sufficient for a determination of its effect on the cells. However, when a substance is placed within the animal, it may be diluted, absorbed, changed by the living cells with which it makes contact, or acted on by various enzymes. Also the cells on which the action of the substance introduced is to be tested are under the influence of factors that limit cell growth and control the regenerative processes of the body. Carrel<sup>1</sup> recognized the differences in the conditions under which cells proliferate within the animal and in tissue cultures, and made experiments in which he applied dead subcutaneous connective tissue to the wounds of dogs. He found that the cicatrization of the wounds was thereby delayed. He attributed the delay to the exclusion of external irritants that in turn stimulate the tissues themselves to produce substances hastening cell regeneration. Dvorak and Byram<sup>2</sup> applied various macerated tissues such as liver, kidney and spleen to wounds of the skin, but found no striking acceleration of healing. Both Carrel and Dvorak and Byram relied on macroscopic examination in a determination of the effects on the wounds. Previously Loeb and Spain<sup>3</sup> had recognized the limitations of the naked-eye appearances in a study of the healing of wounds. Since our purpose was to determine the effect of certain substances on specific cells, it was evident that a histologic study alone would suffice.

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\* From the Department of Pathology, Loyola University School of Medicine.

1. Carrel, A.: *Proc. Inst. Med., Chicago* 8:62, 1930.

2. Dvorak, H. J., and Byram, J. W.: *Proc. Soc. Exper. Biol. & Med.* 27:967, 1930.

3. Loeb, L., and Spain, K. C.: *J. Exper. Med.* 23:107, 1916.

PRODUCTION OF WOUNDS AND PREPARATION OF THE  
MATERIALS APPLIED TO THEM

The cell of the epidermis seemed well adapted for our study. It proliferates in its normal position in the skin and is a highly differentiated cell of specific type. Like other epithelial cells, the epidermoid cell maintains intimate contact with the vascularized membrana propria and, in the case of wounds, with the granulation tissue. Like the other epithelial cells, it thus derives its nutrition from the plasma and is brought under the regulatory mechanism that governs cell growth within the body.

The wounds were produced in young white rats weighing from 150 to 250 Gm. The rats were originally from the Wistar Institute and had been inbred in a more or less haphazard way for a number of years. The technic of producing the wounds was as follows: With the animal under ether anesthesia, the back of the neck was shaved and disinfected with tincture of iodine followed by alcohol. The loose skin was picked up by means of forceps, and two wounds of equal size and identical character were made by cutting through the fold of skin with a sharp 3 mm. leather punch. The two wounds were symmetrically located from 5 to 7 mm. from the roots of the ears. One of the wounds was filled with the macerated tissue or substance to be tested. Both wounds were covered with sterile gauze to a distance slightly beyond their margins. To prevent penetration of the collodion the gauze was impregnated with petrolatum. Dry cotton was spread over this and on the skin beyond. Celloidin was applied to the cotton. When dry, the neck was encircled by a neckband of stiff white cotton duck which was sewed in place. This was made snug, but yet not sufficiently tight to interfere with the circulation. Female rats in individual cages gave almost no trouble after the technic was learned.

The dead macerated tissue fell into two main groups. One was that of macerated epidermis. It was prepared in two ways. After the routine preparation and disinfection of the skin of the tail, the outer epidermal layers were removed by making strokes with a scalpel toward the root of the tail. Such scrapings included not only the cornified layers, but also a large percentage of deeper cells, including those from the rete malpighii. The scrapings were crushed in a mortar with a pestle and finally ground with a rotary motion. A few drops of sterile water were added, and the process was repeated. The mass was kept for twenty-four hours at room temperature and then in the icebox until used. The other preparation was made from 20 mm. embryos in a somewhat different way. The skin was removed from the body and spread on a sterile board with the dermis upward. Much of the myxomatous connective tissue was scraped away with a scalpel. The tissue including the epidermis was then macerated in the mortar.

The second group was that of macerated connective tissue, prepared from the tail tendons. The skin of the tail was slit and stripped from tip to root. The exposed tendons were pulled off and transferred to a sterile mortar. The tendons were first finely minced with scissors and then treated like the epidermis. The consistency was much the same as that of the original tendon. It was kept for twenty-four hours at room temperature before being placed in the icebox. The preparation of other macerated tissues was similar.

At the end of the experimental period, the gauze usually separated with ease from both the experimental and the control wound. In most instances, however, it was removed subsequent to fixation in 10 per cent formaldehyde. The wounds

and the surrounding tissue were removed, spread on blotter paper, to which they were fastened by pins, and then placed in formaldehyde. After fixation, the entire wound was cut into blocks; the blocks were embedded in paraffin and cut into serial sections of 10 microns' thickness. From the ribbon every fifteenth section was selected for staining in hematoxylin and eosin. Measurements were made by a calibrated micrometer ocular. The length of the epithelial tongue, which is the width of the regenerated epidermis, was taken at the center of the wound (middle block). The thicknesses in the tables are averages from the serial sections.

#### RESULTS OBTAINED WITH MACERATED EPITHELIUM

The successful healing of wounds of the skin is dependent on the proliferative activity of the two types of tissue present. Our investigation is concerned especially with the epidermis. The epithelial tongues are the result of epithelial proliferation and were used as a measure of epidermal regeneration. The tongues extended downward to the base

TABLE 1.—*Experiments with Cornified Epithelium*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granu- lation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
91-A.....	3	0.672	1.844	0.264	0.0379	0.0180	0.0030
91-B.....	3	0.073	1.854	0.955	0.0028	0.0187	0.0075
92-A.....	3	0.382	2.096	0.300	0.0248	0.0222	0.0079
92-B.....	3	0.000	2.117	0.697	0.0000	0.0185	0.0075
108-A.....	5	1.270	2.664	0.372	0.0423	0.0415	0.0095
108-B.....	5	0.592	0.774	0.889	0.3978	0.0503	0.0089
109-A.....	5	1.422	2.680	0.692	0.0395	0.0318	0.0090
109-B.....	5	0.475	1.565	0.870	0.0375	0.0500	0.0075
110-A.....	5	0.900	3.108	1.032	0.0310	0.0330	0.0075
110-B.....	5	0.695	1.865	0.625	0.0187	0.0341	0.0075

\* A = experimental wound; B = control wound.

of the wounds and then over the granulation tissue forming there. In this way the proliferating epithelium maintained contact with vascularized connective tissue affording the usual normal nutrition, and at the same time the proliferating cells were in direct contact with the solution products of the macerated epidermis. Both length and thickness of the regenerated epithelium in the wounds filled with macerated epidermis from the rat tail were much greater than in control wounds (table 1). In most instances the epidermal growth was such that it suggested the action of a special formative stimulus derived from the macerated epidermis. However, since the difference between the two wounds might be otherwise explained further experiments were made. That a mechanical effect was being exerted by the filling of the wounds appeared likely from the widths of those of longer duration (table 1). Wounds were therefore filled with a paraffin-petrolatum mixture of a consistency like that of the macerated tissues. These experiments showed the width of the wounds filled with the paraffin-petrolatum mixture to be greater

(table 2). The widths as recorded in the tables were distances between the lateral walls of the wounds. These widths did not correspond to the lengths of the epithelial tongues. Significant contrasts appear in a comparison of tables 1 and 2. In both series of experiments, the granulation tissue was quite active beneath the substances applied. In both, the epithelium extended down the sides and over the base, but beneath the macerated epidermis the extent of the epidermal growth was distinctly greater. Since our problem was concerned especially with the regeneration of the epithelium, a full consideration of all factors involved in

TABLE 2.—*Series of Experiments with Paraffin and Petrolatum Mixture*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
58-A.....	3	0.000	2.870	0.614	0.0000	0.0276	0.0100
58-B.....	3	0.020	1.464	0.772	0.0027	0.0260	0.0100
116-A.....	4	0.431	2.157	0.617	0.0284	0.0403	0.0100
116-B.....	4	0.270	0.744	0.564	0.0180	0.0360	0.0075
112-A.....	5	0.994	0.000	0.576	0.0335	0.0624	0.0100
112-B.....	5	0.422	0.000	0.424	0.0272	0.0324	0.0087

\* A = experimental wound; B = control wound.

TABLE 3.—*Experiments with Fetal Epidermal Tissue*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
140-A.....	3	0.577	2.460	0.210	0.0477	0.0344	0.0100
140-B.....	3	0.079	2.447	0.527	0.0027	0.0211	0.0075
147-A.....	3	0.560	4.433	0.740	0.0292	0.0222	0.0075
147-B.....	3	0.079	2.447	0.527	0.0027	0.0211	0.0075
23-A.....	5	1.200	0.825	0.800	0.0566	0.1950	0.0125
23-B.....	5	0.215	0.430	0.600	0.0338	0.0744	0.0100

\* A = experimental wound; B = control wound.

wound healing is unnecessary. Our examination of the normal control wounds confirms the observations of Akaiwa<sup>4</sup> in the rat that contraction plays an insignificant part, and that the healing is accomplished by the regeneration and movement of epithelium and by the formation of granulation tissue. Akaiwa further pointed out that the thickness of the stratum germinativum corresponds to the proliferative activity and energy of cell movements.

Confirmation of the stimulant action of macerated epidermis on epidermal cells was sought by employing fetal epidermis, which was prepared in the manner previously described. Again the epidermis was much more active than that of the control wounds (table 3). This

4. Akaiwa, H.: J. M. Research 40:371, 1919.



preparation stimulated not only the epidermis, but also the production of granulation tissue, which was the greatest seen in the entire series of experiments. In this connection it is significant that the preparation contained considerable myxomatous dermis and subcutaneous tissue, while the scrapings from the adult tail contained little connective tissue.

A commercial keratin (Merck) was brought to the consistency of the macerated tissue and applied to the wounds (table 4). The length of the epithelial tongue and the width of the defect in the wounds to which the keratin was applied indicate that it was without effect or that it

TABLE 4.—*Experiments with Commercial Keratin*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granu- lation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
126-A.....	4	0.270	4.200	0.520	0.0337	0.0225	0.0075
126-B.....	4	0.347	4.080	0.450	0.0286	0.0250	0.0075
125-A.....	5	0.250	3.780	1.850	0.0159	0.0194	0.0100
125-B.....	5	0.400	0.000	0.253	0.0242	0.0200	0.0083

\* A = experimental wound; B = control wound.

TABLE 5.—*Experiments with Ether and Alcohol Treated Cornified Epidermis*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granu- lation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
128-A.....	4	0.203	2.935	0.300	0.0172	0.0153	0.0075
128-B.....	4	0.417	0.833	0.613	0.0201	0.0314	0.0075
127-A.....	5	0.205	3.195	0.550	0.0144	0.0212	0.0100
127-B.....	5	0.463	0.460	0.633	0.0237	0.0279	0.0100

\* A = experimental wound; B = control wound.

retarded epidermization somewhat. Since the source and composition of this product were unknown, the results obtained are not offered as proof that rat keratin would be without effect.

*Alcohol-Ether Treated Epidermis.*—Scrapings from the rat tail were macerated in the usual way and then ground with absolute alcohol, which was decanted, and the operation twice repeated. The residue was then treated with ether in the same fashion. The product was a whitish powder, which was brought to the desired consistency by the addition of a few drops of sterile distilled water. Two obvious changes were brought about, which consisted of the removal of lipoids and the coagulation of protein. Epidermal regeneration in this series (table 5) was not increased. The length and the thickness of the tongues were less than in the control wounds.

One attempt was made to extend the experiments to an unrelated epithelial tissue, the liver. It was crushed after washing out the blood while in situ and applied in the usual way. The proliferative activity of the epidermis was not much affected. The length of the tongues was somewhat greater than in the control wounds, but their thickness was variable (table 6).

#### RESULTS OBTAINED WITH MACERATED CONNECTIVE TISSUE

As in the preceding experiments, with macerated connective tissue the cells of the stratum germinativum adjacent to the wound increased

TABLE 6.—*Experiments with Liver Tissue*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
137-A.....	3	0.380	2.853	0.327	0.0225	0.0437	0.0075
137-B.....	3	0.337	2.433	0.163	0.0150	0.0533	0.0075
138-A.....	4	0.523	0.523	0.600	0.0316	0.0304	0.0083
138-B.....	4	0.315	2.620	0.090	0.0309	0.0325	0.0088

\* A = experimental wound; B = control wound.

TABLE 7.—*Experiments with Connective Tissue*

Experiment*	Days	Length of Epithelial Tongue, Mm.	Width of Defect, Mm.	Thickness of Granulation Tissue, Mm.	Cell Thickness of Epithelium		
					Tip of Tongue, Mm.	Root of Tongue, Mm.	Old Epidermis, Mm.
42-A.....	3	0.010	3.836	0	0.0026	0.0179	0.0075
42-B.....	3	0.000	2.308	0.424	0.0000	0.0088	0.0075
53-A.....	3	0.172	3.100	0.332	0.0240	0.0481	0.0102
53-B.....	3	0.010	2.416	0.764	0.0030	0.0227	0.0100
102-A.....	4	0.299	2.280	0.263	0.0185	0.0265	0.0079
102-B.....	4	0.417	0.833	0.613	0.0201	0.0314	0.0075
104-A.....	4	0.073	2.520	0.790	0.0146	0.0241	0.0095
104-B.....	4	0.417	0.833	0.613	0.0201	0.0314	0.0075
96-A.....	5	0.174	3.666	0.458	0.0076	0.0496	0.0100
96-B.....	5	0.840	0.500	0.860	0.0147	0.0284	0.0100
97-A.....	5	0.183	3.208	0.688	0.0100	0.0320	0.0075
97-B.....	5	0.510	0.000	1.050	0.0100	0.0354	0.0100

\* A = experimental wound; B = control wound.

in size and moved toward the margin of the wound soon after its production. These changes and the increase in mitoses contributed to the thickening and extension of the epithelium. In the control wounds, the average thickness of the basal layer was 0.0211 mm. on the third day, 0.0314 mm. on the fourth and 0.0377 mm. on the fifth, while the normal thickness was about 0.0075 (table 7). The increase in the length of the epithelial tongues on which epidermization of the wound depends started on the fourth day in both experimental and control wounds. In the control wounds there was a progressive daily increase, while in the experimental ones there was much individual variation. In all, the

epithelium showed a tendency to extend over the surface of the dead tissue rather than beneath it. After the fourth day the lagging of epidermization in these experiments was evident. We were able therefore to confirm the observations of Carrel,<sup>1</sup> who covered the wounds with dead subcutaneous connective tissue.

#### COMMENT

Dead macerated epidermis, both adult and fetal, promotes the proliferation of the epidermal cells. The stimulant action is local, since control wounds only 2 cm. distant do not show it. That the increase in growth is not mechanical and due to a protective influence of an inert substance is shown by its absence in the wounds filled with the paraffin-petrolatum mixture. The evidence is that from the macerated epidermis undergoing local disintegration there is liberated a formative stimulus which, so far as one may judge from our experiments, is specific for the epidermal cells. The soft macerated epidermis physically offers little resistance to the extension of cells into it, and yet the epidermis extends beneath it. This behavior is seen also in the paraffin-petrolatum wounds and no doubt is brought about by the concentration of nutrition in this situation. Although the macerated epidermis stimulates epidermal regeneration, it appears not to afford complete nutriment to the proliferation of epidermoid cells. The growth promoting substance is not present in other macerated tissues, and it is destroyed by alcohol ether extraction. Baker and Carrel<sup>5</sup> found that the addition of primary derivatives of protein to the media accelerated the growth of cultures of guinea-pig skin, and Carrel<sup>1</sup> mentioned that these substances are normally present in organs, and that they may be manufactured by leukocytic ferments from cell debris and coagulated proteins. Hammett<sup>6</sup> advanced the hypothesis that relatively simple substances containing the sulphydryl group, such as glutathione, excite cells to proliferate. Voegtlin and Chalkley<sup>7</sup> demonstrated the stimulant action of this tripeptide on *Amoeba proteus*. Girand and Bulliard<sup>8</sup> found cornified epidermis to be relatively poor in glutathione, while the liver is rich in this substance. So far as we can determine, our results give little information in regard to the part of the epithelial cell that acts as the stimulant, but they tend to show that the stimulus for the epidermoid cell is peculiar to the macerated epidermis and is not of a general nature as are the polypeptide derivatives of fibrin.

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5. Carrel, A., and Baker, L. E.: *J. Exper. Med.* **46**:503, 1926. Baker, L. E., and Carrel, A.: *ibid.* **47**:353, 1928.

6. Hammett, F. S.: *Protoplasma* **7**:20, 1929.

7. Voegtlin, C., and Chalkley, H. W.: *Pub. Health Rep.* **45**:30, 1930.

8. Girand, A., and Bulliard, H.: *Compt. rend. Soc. de biol.* **98**:500, 1928.

We found, as did Carrel, that the healing of a wound is retarded by filling it with macerated connective tissue. The histologic study of our wounds revealed a tendency of the epidermis to extend over this dead tissue to a point where lack of nutrition halted the growth. In several instances, the dead tissue, more tenacious than the other macerated tissues used, offered mechanical obstruction to the extension of the epidermis in any other direction. We are therefore inclined to favor the view that the delay in cicatrization was due to mechanical and nutritional rather than to chemical factors. A close study was made of these wounds to determine whether the macerated tendon might stimulate fibroblastic proliferation, but no consistent evidence of this was obtained. On the other hand, the fetal skin that contained immature connective tissue unquestionably stimulated the formation of angiofibroblastic tissue and produced the most abundant granulation tissue seen.

#### CONCLUSIONS

Macerated homologous epidermis applied to wounds stimulates epidermal regeneration.

Evidence was obtained which indicates that the stimulation is a chemical action and not the indirect result of a change in physical environment.

Failure of other macerated tissues to act similarly is suggestive of the presence in the epidermis of a growth-promoting substance specific for the epidermoid cell.

# General Review

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## AN ASPECT OF INFLAMMATION IN RELATION TO IMMUNITY \*

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The experimental studies on inflammation began about seventy years ago, when Cohnheim<sup>1</sup> demonstrated the migration of leukocytes from the blood into the tissue spaces. This, according to Cohnheim, was primarily the result of injury to the walls of the capillaries with the consequent passive passage of plasma constituents and blood corpuscles into the tissue spaces. Metchnikoff<sup>2</sup> regarded inflammation as a phagocytic reaction to injurious material that had penetrated the tissues. The leukocytes either came from the blood or were modified cells of the tissues at the site of the injury:

L'inflammation doit donc être envisagée dans son ensemble comme une réaction phagocytaire de l'organisme contre les agents irritatifs, réaction qui tantôt s'accomplit par les phagocytes mobiles seuls, tantôt avec le concours des phagocytes vasculaires ou celui du système nerveux. (One has to imagine inflammation as a phagocytic reaction of the organism against the irritating agents, a reaction which sometimes is accomplished by the mobile phagocytes alone and sometimes with the aid of the vascular phagocytes or those of the nervous system.)

Inflammation was not regarded by Metchnikoff as primarily a lesion of the vascular walls in the sense of Cohnheim, but rather as an adaptive reaction on the part of phagocytic cells for the express purpose of protecting the organism as a whole. I need not dwell on these early views, except to note that the migration of leukocytes at the point of injury was established as the essential phase of the inflammatory reaction. Other elements of the reaction, such as would accompany an outpouring of plasma into the tissue spaces, were more or less relegated to the background. Some of the early writers, however, pointed out the importance of deposits of fibrin in certain types of inflammation. The observations of Adami<sup>3</sup> are significant in this connection:

Even when inflammation (as in pericarditis) affects the whole extent of a serous cavity, the layer of fibrin acts as a protective coat closing the lymphatic

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\* From the Department of Pathology, Harvard Medical School.

1. Cohnheim, J.: *Virchows Arch. f. path. Anat.* **40:1**, 1867; *Vorlesungen*, ed. 2, Leipzig, 1882.

2. Metchnikoff, E.: *Leçons sur la pathologie comparée de l'inflammation*, Paris, G. Masson, 1892.

3. Adami, G. J.: *Inflammation*, London, The Macmillan Company, 1909.

"stomata" hindering the free absorption of the morbid material by the lymph and blood vessels, and filtering bacteria out of such fluid as does find its way through to the tissues beneath.

Opie<sup>4</sup> defined inflammation as a process by means of which cells and serum accumulate about an injurious substance and tend to remove or destroy it.

In the usual textbook description of the inflammatory reaction, except for an occasional reference to the importance of the accumulation of serum at the site of inflammation, the predominant rôle of the leukocytes completely overshadows the activities of all other elements.

In the last few years evidence has been brought forward to show that the inflammatory reaction plays a definite rôle in localizing the irritating factor, or at least limiting its dissemination, whether it is a bacterial or a chemical irritant. This capacity, which may appropriately be termed fixation, contributes a further significance to inflammation in relation to immunity. By delaying the dissemination of obnoxious substances into the circulating blood stream, the nonspecific as well as the specific inflammatory reaction protects the organism as a whole at the expense of local injury. This view of inflammation has gained considerable ground in the last few years. Recent analyses have demonstrated that fixation at the site of inflammation is due to mechanical obstruction caused by a network of fibrin and to occlusion of lymphatic vessels.<sup>4a</sup> Studies on the time relationship have shown that fixation is the initial phase in the development of the inflammatory reaction, even preceding the migration of leukocytes. This is doubtless due to injury of the capillaries causing an increase in the permeability of their walls, which results in an outpouring of fibrinogen into the tissue spaces. Pathologists are gradually reverting to the concept of Cohnheim, namely, that in inflammation the primary injury is to the wall of the capillaries. Furthermore, the early development of thrombi in the lumen of lymphatics is doubtless the result of severe initial injury to the lymphatic wall itself in an inflamed area. In view of present evidences, as will be pointed out, one can ascribe a definite function to the early formation of fibrin at the site of inflammation. Fixation plays a definite rôle in inflammation and may be considered in the same light as the migration of leukocytes and the stage of fibrous repair that follow. Chronologically the inflammatory reaction may be broadly divided, therefore, into three phases, which, it is understood, may overlap: (1) fixation, (2) emigration of leukocytes and (3) repair.

The last two phases are well known to pathologists and will not be dwelt on further. The purpose of this review is to describe the evidences that recently have accumulated which show that with the initial increase

4. Opie, E. L.: *Arch. Int. Med.* **5**:541, 1910; *J. Immunol.* **17**:329, 1929.

4a. Menkin, V.: *J. Exper. Med.* **53**:171 and 179, 1931; *Arch. Int. Med.*, in press.

in the permeability of the capillaries and the passage of plasma constituents into tissue spaces the inflammatory reaction is capable of rapidly circumscribing the irritant and thus allowing a definite interval of time for the leukocytes to assemble for phagocytosis. Fixation, as the initial phase of the inflammatory reaction, therefore becomes of distinct importance as a mechanism in immunity.

#### FIXATION OF BACTERIA BY THE NONSPECIFIC INFLAMMATORY REACTION

The dissemination of foreign substances from the site of injection into the lymphatics and into the blood stream has been studied by numerous investigators. Muscatello<sup>5</sup> showed that carmine and various other inert particles, when injected into the peritoneal cavity, reached the anterior mediastinal lymph nodes very rapidly. Noetzel<sup>6</sup> injected bacteria (*Bacillus pyocyaneus*) into the knee joints of rabbits; from five to ten minutes later he was able to demonstrate the presence of the organisms in the inguinal, crural and lumbar lymph glands. Buxton<sup>7</sup> found that typhoid bacilli, within a few minutes after their injection into the peritoneal cavity, appeared in great numbers in the blood stream. Wells and Johnstone<sup>8</sup> showed that the absorption of bacteria from the peritoneal cavity takes place through lymphatic vessels.

Does the inflammatory reaction retard the dissemination of micro-organisms from the site of inflammation? The studies of Issayeff<sup>9</sup> are interesting in this connection. He showed that the peritonitis induced by a variety of sterile irritants, such as foreign blood serum, bouillon or normal salt solution, temporarily increased resistance to subsequent intraperitoneal injection of bacteria. Pawlowsky<sup>10</sup> repeated the observations of Noetzel and demonstrated the presence of staphylococci in the blood and organs of guinea-pigs from twenty-four to forty-eight hours after inoculation of the knee joint. If before inoculation an acute inflammation of the knee joint had been produced by the injection of a sterile irritant, such as turpentine, alcohol or quinine solution, the dissemination of the micro-organisms was either inhibited or wholly prevented.

Rivers and Tillett<sup>11</sup> confirmed these studies by finding that beef broth injected into the skin of rabbits protected against streptococci inoculated twenty-four hours later. No retardation was observed when

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5. Muscatello, G.: Virchows Arch. f. path. Anat. **142**:327, 1895.

6. Noetzel, W.: Beitr. z. klin. Chir. **51**:740, 1906.

7. Buxton, B. H.: J. M. Research **16**:17, 1907.

8. Wells, H. G., and Johnstone, O. P.: J. Infect. Dis. **4**:582, 1907.

9. Issayeff: Ztschr. f. Hyg. u. Infectiönskr. **16**:287, 1894.

10. Pawlowsky, A. D.: Ztschr. f. Hyg. u. Infectiönskr. **62**:433, 1909.

11. Rivers, T. M., and Tillett, W. S.: J. Exper. Med. **41**:185, 1925.

the micro-organisms were inoculated simultaneously with the beef broth. Mallory and Marble<sup>12</sup> concluded that the local resistance produced by filtrates of staphylococci was due to local inflammation and was therefore not specific. It is to be recalled that Besredka<sup>13</sup> advocated the therapeutic use of such filtrates. Opie<sup>14</sup> showed that acute inflammation of the peritoneal cavity caused by aleuronat retarded the rush of injected hemolytic streptococci from the peritoneal cavity into the circulating blood and after twenty-four hours completely prevented it. By a culture method I<sup>15</sup> demonstrated that *B. prodigiosus* injected into an inflamed area was fixed in situ and failed to disseminate into the tributary lymphatics.

Some authors believe that in inflammation the large mononuclear phagocytes are responsible for protection. Nakahara<sup>16</sup> injected olive oil into the peritoneal cavity and showed that after forty-eight hours an exudate was formed containing from 65 to 85 per cent macrophages. At this stage the resistance of mice to *B. coli* introduced into the peritoneal cavity was definitely increased. Gay and Morrison<sup>17</sup> studied the changes in resistance to intrapleural inoculation of streptococci after a sterile inflammation had been previously caused by various irritants. They contended that the increased resistance to the pathogenic micro-organism resulted from the accumulation of clasmotocytes. This interpretation was questioned by Opie,<sup>14</sup> who maintained that no data had been offered that determined the rôles of polymorphonuclear leukocytes, mononuclear cells, serum or indeed increased permeability of blood vessels in overcoming the injurious effects of sterile irritants or of bacteria. Gay and Clark<sup>18</sup> in answer to these criticisms produced some evidence showing that perhaps the polymorphonuclear leukocytes were relatively inactive in increasing the resistance of their animals. However, the increased resistance to virulent bacteria brought about by previously "preparing" a cavity with a sterile irritant is evident only when the inflammatory reaction has been going on in the cavity for from forty-eight to seventy-two hours. Local fixation of substances at the site of inflammation, as will presently be shown, can be demonstrated a relatively short time (sometimes after only thirty minutes) after the introduction of the inflammatory irritant.<sup>19</sup> For this reason the increased resistance to intrapleural inoculation of streptococci found by Gay and

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12. Mallory, T. B., and Marble, A.: J. Exper. Med. **42**:465, 1925.

13. Besredka, A.: Immunisation locale, Paris, Masson & Cie, 1925.

14. Opie (footnote 4, second reference).

15. Menkin, V.: J. Exper. Med. **53**:647, 1931.

16. Nakahara, W.: J. Exper. Med. **42**:201, 1925.

17. Gay, F. P., and Morrison, L. F.: J. Infect. Dis. **33**:338, 1923.

18. Gay, F. P., and Clark, A. R.: Proc. Soc. Exper. Biol. & Med. **27**:995, 1930.

19. Menkin, V.: J. Exper. Med. **50**:171, 1929.



his collaborators probably has nothing to do with the initial phase of local fixation in inflammation, but is evidently referable to a later phase of the inflammatory reaction.

#### FIXATION OF BACTERIA BY THE SPECIFIC INFLAMMATORY REACTION

Koch<sup>20</sup> described the immune reaction that takes place when a tuberculous guinea-pig is reinoculated with either living or dead tubercle bacilli. This reaction to reinfection is known as the Koch phenomenon and forms probably the most important single principle in the understanding of immunity in tuberculosis. A part of Koch's original description follows (cited from a translation by Pottenger<sup>21</sup>):

When one inoculates healthy guinea-pigs with a pure culture of tubercle bacilli the inoculation wound closes up and apparently heals within a few days. In the course of ten to fourteen days, however, a hard nodule appears, which breaks down and forms an ulcer. This remains until the death of the animal. It is quite different, however, when a guinea-pig that is already tuberculous is inoculated. Animals that have been inoculated from four to six weeks previously are best suited to this purpose. In one such animal, however, the inoculation did not heal in the beginning, but small nodules appeared, and within one or two days showed characteristic changes about the point of inoculation. Around the point of inoculation it became hard and took upon itself a darker color, which did not confine itself to the immediate point of inoculation but extended to the surrounding tissues for a distance of 0.5 to 1 cm. When the succeeding dose is administered it shows very distinctly that the skin showing the alteration is necrotic; and it is eventually cast off, a small superficial ulceration remaining, which usually heals quickly and permanently without the regional lymph-glands becoming involved. . . .

After these characteristic effects had been discovered, I followed them in every direction and I found that pure cultures of tubercle bacilli that had been killed, ground up, and mixed with water could be injected in large amounts subcutaneously in healthy guinea-pigs without producing anything beyond a local suppuration. Tuberculous guinea-pigs, on the contrary, were killed by the injection of very small amounts of such cultures of dead bacilli within six to forty-eight hours after administration of the dose. A dose that is not sufficiently large to kill an animal often produces an extensive necrosis of the skin in the neighborhood of the site of injection.

Pirquet called the altered response of tuberculous tissue to reinfection "allergy." The reaction to tuberculin, which may be regarded as a manifestation of the Koch phenomenon by means of tuberculo-protein, is histologically an acute inflammation, as pointed out by Opie.<sup>14</sup> There is an abundant accumulation of serum and polymorphonuclear leukocytes during the first forty-eight hours. Polymorphonuclear leukocytes are finally displaced by mononuclear cells, partly at least as the result of phagocytosis.

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20. Koch, R.: *Deutsche med. Wchnschr.* **17**:101, 1891.

21. Pottenger, F. M.: *Tubercle* **10**:409, 1929.

In 1916, Kraus<sup>22</sup> mentioned how the work of Pawlowsky and Issayeff cited by Opie (1910)<sup>4</sup> had suggested to him the possibility that specific immunity to reinfection in tuberculosis might be a function of allergy in that the inflammation accompanying the latter prevents the spread of reinfecting bacilli. Later, Kraus<sup>23</sup> put the case more concretely as follows: "The tuberculous animal responds with exaggerated or overreaction to irritation and throws up a barrier against the bacilli much more actively than normally and before the bacilli can get under way."

Kraus and Willis<sup>24</sup> showed experimentally the extent of the retardation in infected animals. Willis<sup>25</sup> studied the dissemination of tubercle bacilli from the site of cutaneous inoculation, and observed that in reinjected guinea-pigs the spread of tubercle bacilli from the site of inoculation was retarded, whereas in normal animals the organisms passed readily to the regional lymph nodes. Both Kraus and Willis laid emphasis on the mechanical barrier set up against the outward movement of the deposited bacilli. "We believe, therefore, that specific tuberculo-immunity occurs through a fixation of germs that results from the operation of the allergic reaction. An almost immediate inflammatory outpouring hems in the bacilli more or less effectively and thus delays or prevents their spread, which is so facile and rapid in the non-tuberculous, non-allergic animal."<sup>26</sup>

Rich and McCordock<sup>27</sup> attacked the hypothesis of Kraus and Willis, largely because of certain apparent inconsistencies in the arguments of the latter. In the first place, Rich and McCordock pointed out that, although it was assumed that inflammation per se can limit the immediate spread of micro-organisms, there was not the slightest proof of this. "And until we have more precise information on the question it must be remembered that the inoculation results in the experiments of Kraus and Willis might be interpreted on the basis of the well established fact that there occurs a much greater destruction of bacilli in immune animals than in normal ones." An interpretation of this sort would be difficult to accept in view of the facts obtained. Willis<sup>25</sup> demonstrated that the regional lymph nodes in immune animals were infected at the end of two weeks after cutaneous inoculation. If the apparent retardation of bacilli at the site of inoculation was really due to their destruction in situ by a subtle immunologic mechanism, their

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22. Kraus, A. K.: *Tr. Nat. A. Prev. Tuberc.* **12**:253, 1916.

23. Kraus, A. K.: *Am. Rev. Tuberc.* **1**:65, 1917.

24. Kraus, A. K., and Willis, H. S.: *Tr. Nat. A. Prev. Tuberc.* **20**:277, 1924.

25. Willis, H. S.: *Am. Rev. Tuberc.* **11**:427 and 439, 1925.

26. Kraus, A. K.: *Am. Rev. Tuberc.* **11**:343, 1925.

27. Rich, A. R., and McCordock, H. A.: *Bull. Johns Hopkins Hosp.* **44**:273, 1929.

appearance after a latent interval of two weeks in the regional lymph nodes would be difficult to explain. Furthermore, I<sup>15</sup> recently demonstrated that an acute inflammatory reaction caused by a nonspecific irritant prevented for at least several hours the dissemination of *B. prodigiosus* to the tributary lymph nodes. A distinctly larger number of micro-organisms were recovered from the site of inflammation than from the corresponding normal control area. These experiments demonstrated that an acute inflammation per se retards the dissemination of bacteria into the regional lymphatics.

Kraus and Willis<sup>28</sup> showed that reinfection with tubercle bacilli in areas where an allergic inflammation had been induced by tuberculin caused more widespread tuberculosis than that seen in reinfected control animals that had received no tuberculin. Their observations have been brought forward as evidence that the allergic inflammation does not retard the dissemination of bacilli. Such an interpretation of these particular experiments, however, is open to some doubt. It is possible, in view of recent observations that I made<sup>29</sup> on the accumulation of foreign proteins in inflamed areas, that preliminary treatment of infected animals with tuberculin might cause an accumulation from the blood stream of tuberculo-protein in various inflamed tuberculous lesions of the body, which by its presence there would intensify the preexisting local inflammatory reaction.

The view that resistance may be at times independent of allergy and thus be referable entirely to a subtle immunologic mechanism is not questioned. Zinsser and his collaborators<sup>30</sup> expressed the view that "the fundamental biological significance of bacterial allergy is an increased specific adjustment of the tissues for response to the stimulus of infection, and a consequently enhanced capacity for the rapid mobilization of a protective mechanism." The fundamental point, however, is this: When an allergic inflammation develops as a result of reinfection, does the inflammatory reaction play a definite rôle in the mechanism of immunity by retarding the dissemination of bacteria? Evidences cited and some that are to follow confirm the view that an acute inflammation either of specific origin or caused by a nonspecific irritant definitely delays the spread of injurious substances from the site of inflammation.

Wadsworth<sup>31</sup> injected virulent pneumococci into the tracheas of normal animals and thereby caused fatal bacteremia which in some cases was associated with bronchopneumonia. When immunized animals were

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28. Kraus, A. K., and Willis, H. S.: *Am. Rev. Tuberc.* **4**:563, 1920.

29. Menkin, V.: *J. Exper. Med.* **52**:201, 1930.

30. Zinsser, H.; Ward, H. K., and Jennings, F. B., Jr.: *J. Immunol.* **10**:719, 1925.

31. Wadsworth, A.: *Am. J. M. Sc.* **127**:851, 1904.

treated in the same manner, there was a diffuse exudation comparable with lobar pneumonia, but no invasion of the blood stream. Cecil and Blake<sup>32</sup> also found that the incidence of invasion of the blood stream was less in monkeys vaccinated against pneumonia induced by intratracheal inoculation than in nonvaccinated animals. Cannon and Pacheco<sup>33</sup> described histologic studies of the skin and subcutaneous tissues of the abdominal walls of normal guinea-pigs and of animals previously immunized by intracutaneous injections of a staphylococcal vaccine. All the animals were infected by the intracutaneous injection of a live virulent culture of *Staphylococcus aureus*. In the normal animals the staphylococci disseminated throughout the subcutaneous tissues as a cellulitis. In previously immunized animals, on the other hand, the staphylococci tended to remain localized near the site of inoculation. These investigators expressed the belief that the immunity secured was predominantly cellular, with the tissue macrophages playing the dominant part. Localization of the micro-organisms was also reinforced to some extent by the action of agglutinating or of opsonizing antibodies.

#### FIXATION OF FOREIGN PROTEINS BY THE SPECIFIC INFLAMMATORY REACTION

Evidence has been cited showing that immunization retards the dissemination of bacteria from the site of injection, where specific inflammation occurs. Extensive studies by Opie<sup>34</sup> demonstrated that a foreign protein injected into the skin of a sensitized animal is fixed at the site of injection. This fixation is accompanied by an acute inflammatory reaction (Arthus' phenomenon).

When a small quantity of a foreign protein, such as horse serum or egg white, is injected into a normal rabbit, it is demonstrable by the precipitin reaction in the blood of that animal for a period of from seven to nine days. Opie<sup>35</sup> showed that with continued immunization injected proteins exhibit a decreasing tendency to find their way into the blood. After a few immunizing injections they can be found in the blood serum in only small quantities and for but a short time. After the sixth or seventh injection well immunized animals reveal no trace of the proteins in the circulating blood.

Opie undertook to determine what happened to a foreign protein at the site of injection in the normal and in the immune animal. The cutaneous site of injection was excised, extracted with normal salt solution and tested by the precipitin reaction for the presence of the foreign

32. Cecil, R. L., and Blake, F. S.: J. Exper. Med. **31**:519, 1920.

33. Cannon, P. R., and Pacheco, G. A.: Am. J. Path. **6**:749, 1930.

34. Opie, E. L.: J. Immunol. **9**:231, 1924; J. Exper. Med. **39**:659, 1924; footnote 4, second reference.

35. Opie (footnote 34, second reference).

protein. The concentration of the foreign protein extracted was estimated by comparison with solutions of known protein content. Crystalline egg albumin injected into a normal rabbit was found in small concentration on the first and second day (1:250 and 1:500, respectively). On the third day it had disappeared from the site of injection. In an immunized animal, on the other hand, the concentration of the protein was much greater at the site of injection than in a corresponding normal area. In one experiment the foreign protein was recovered on the first day in a concentration of 1:80 at the periphery of the inflamed area; on the second day in a concentration of 1:50; it did not disappear until the fifth day.

In this way Opie demonstrated that in the immunized animal, fixation of the antigen at the site of injection prevented its dissemination throughout the body. Opie<sup>36</sup> expressed the opinion that specific precipitation had a part in this fixation. The precipitate formed by the contact of antigen and antibody has a strong attraction for polymorphonuclear leukocytes of the blood and, according to Opie, is doubtless digested and destroyed by these cells. In a later study in Professor Opie's laboratory I<sup>29</sup> demonstrated that a nonspecific acute inflammatory reaction is capable of arresting a foreign protein at the site of injection. For this reason it is probable that the fixation of antigen in Arthus' phenomenon is largely due to the acute inflammatory reaction brought about by the contact of antigen and antibody in the tissues. Opie<sup>36</sup> pointed out that it is unnecessary to assume that the tissues have undergone any essential alteration as the result of sensitization. "Acute inflammation in response to a specific irritant, namely, Arthus' phenomenon, occurs when the antigen concerned and its antibody meet within the tissues." This conclusion was reached by reversing the usual procedure. A normal animal received 10 cc. of horse serum intravenously. On the following day 0.5 cc. of strong anti-horse serum from an immunized rabbit was injected into the skin. Extensive inflammatory edema (Arthus' phenomenon) occurred at the site of injection, whereas none followed the injection of normal rabbit serum. Opie viewed the anaphylactic inflammation in which the antigen is fixed at the point of entry as an apparent paradox between susceptibility to injury and the resistance of immunity. With the anaphylactic inflammation the vital organs are protected at the expense of local injury. In a recent review Nordmann<sup>37</sup> criticized Opie's interpretation of Arthus' phenomenon. This author maintained that ordinary inflammatory phenomena such as are experimentally produced by the simultaneous injection of two serums into a normal animal do not show the gradually increasing severity of

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36. Opie, E. L.: *Tubercle* 7:23, 1925.

37. Nordmann, M.: *Physiol. Rev.* 11:41, 1931.

symptoms that is the characteristic feature of the Arthus' phenomenon. It is clear, however, that this gradual change in severity of symptoms in anaphylactic inflammation may be to some extent (though not entirely) correlated with the increase in precipitin during the process of immunization. Nordmann insisted that physiologic alterations play an important rôle in the development of Arthus' phenomenon: "We conclude therefore that the cause of the final necrosis following the later injections in the Arthus experiment is due to the ability of the arteries to become accustomed to the serum and that in consequence of this adaptation they are less and less altered by additional injections of the antigen." Although it is perfectly natural that physiologic changes should accompany the anaphylactic inflammation, it is doubtful whether this author has demonstrated that the particular changes in the arteries influence the development of the anaphylactic inflammatory reaction. The studies of Opie indicate that this reaction is brought about by the contact of antigen and antibody at the site of injection. An inflammatory reaction of unusual intensity occurs, and this reaction doubtless brings to the site of inflammation plasma containing more precipitin. This view is in agreement with facts recently demonstrated by myself,<sup>39</sup> namely, that foreign proteins from the circulating blood stream accumulate in an inflamed area. The initial anaphylactic inflammation is intensified, and this may account for the gradual increase in the severity of the symptoms of Arthus' phenomenon.

#### ACCUMULATION AND FIXATION OF FOREIGN SUBSTANCES AT SITE OF INFLAMMATION

In an inflamed area the normal functional equilibrium between cells, intercellular fluids and blood is doubtless profoundly modified. Local physiologic changes are involved, affecting the permeability of the capillaries, the rate of the blood flow and the balance of the body fluids. The increase in the permeability of the capillaries is shown by the local edema. The proteins of the plasma to a varying extent pass out into the tissue spaces, disturbing, doubtless, the osmotic relationship between the fluids of the tissues and the plasma of the blood. About thirty years ago Adler and Meltzer<sup>38</sup> concluded that the passage of fluids from tissue spaces into lymphatics probably depended on osmosis, perhaps assisted to some degree by filtration. Starling<sup>39</sup> pointed out that capillary filtration was the resultant of two forces: capillary pressure, on the one hand, and the osmotic pressure exerted by the plasma colloids, on the other. Recently this conception has been confirmed experimentally by the work of Landis,<sup>40</sup> who showed by measuring directly intracapil-

38. Adler, I., and Meltzer, S. J.: *J. Exper. Med.* **1**:482, 1896.

39. Starling, E. H.: *J. Physiol.* **19**:312, 1895-1896.

40. Landis, E. M.: *Am. J. Physiol.* **81**:124, 1927.

lary pressure that the rate of filtration depends on the difference between intracapillary pressure and the osmotic pressure of the plasma colloids. By this direct method he found that whereas filtration occurs at the arterial ends of capillaries, absorption from tissue spaces takes place at the venous ends. Churchill, Nakazawa and Drinker,<sup>41</sup> however, demonstrated that, in the frog, at least, the osmotic pressure of the plasma proteins is lower than indicated by the figures given by White<sup>42</sup> and used by Landis in his interpretations. For this reason these investigators thought it safer to conclude that there exists a filtration of fluid throughout the system. Rous and his collaborators<sup>43</sup> cast some doubt on the results obtained by Landis. They showed that a vital dye injected intravenously diffuses first into the tissue spaces on the venous ends of the capillaries. These investigators maintained that there is a mounting gradient of permeability throughout the length of the capillary. This was demonstrated in a variety of organs in different animals. Furthermore, when they made the same observations as Landis, with only slight modifications, on injection of a vital dye into the vessels of the mesentery of the frog, they were unable to confirm his findings of increased filtration of dye at the arterial ends of the capillaries.

The normal equilibrium of the filtration through the wall of the capillary, however, is doubtless modified in inflammation by the passage of proteins from the plasma into the tissue spaces. If the passage of lymph from tissue spaces into afferent lymphatic vessels depends largely on an osmotic equilibrium, it is probable that the accumulation of plasma proteins in the tissue spaces modifies and retards the flow of lymph into the afferent lymphatic vessels. Schade and Menschel<sup>44</sup> found that in inflamed areas, especially in those with suppuration, the accumulation of products of tissue degeneration may become so great that the osmotic pressure is raised as high as 11 atmospheres. An inflamed area can be considered as shunted off from the rest of the organism. It has its own metabolism, its own hydrogen ion concentration, and, as was just pointed out, its own modified circulation.

I studied the behavior of foreign substances injected into an inflamed area by direct observation of the tributary lymphatic nodes and vessels draining the area. After some preliminary experimentation with the nucleated corpuscles of fowls, a vital dye, trypan blue, was tried and found to be satisfactory.<sup>10</sup> If trypan blue is injected into the normal

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41. Churchill, E. D.; Nakazawa, F., and Drinker, C. K.: *J. Physiol.* **63**:304, 1927.

42. White, H. L.: *Am. J. Physiol.* **68**:523, 1924.

43. Rous, P.; Gilding, H. P., and Smith, F.: *J. Exper. Med.* **51**:807, 1930. Smith, F., and Rous, P.: *J. Exper. Med.* **53**:195, 1931. Rous, P., and Smith, F.: *J. Exper. Med.* **53**:219, 1931.

44. Schade, H., and Menschel, H.: *Ztschr. f. klin. Med.* **96**:279, 1923.

subcutaneous tissue of the foreleg of a rabbit, within from twenty to thirty minutes the dye stains the tributary afferent lymphatic vessels, the lymph node and the efferent lymphatic vessel. If, on the other hand, the dye is injected into a similar area in which an inflammatory reaction has been produced some time before by a sterile irritant, the dye does not appear in the tributary lymphatics; the afferent lymphatic vessels, the lymph node and the efferent lymphatic vessel remain colorless. The dye is evidently retained in the inflamed area. The fixation of the dye takes place soon after the injection of the irritant, being observed in some instances when the dye is injected thirty minutes after the injection of the irritant.

Since trypan blue tends to remain in situ when injected directly into the inflamed area, an attempt was made, at the suggestion of Prof. Eugene L. Opie, to determine whether dye injected into the blood stream would accumulate at the site of inflammation. MacCurdy and Evans<sup>45</sup> pointed out that the normal brain and spinal cord remain free from dye injected intravenously, but that areas of damage, such as softening or inflammation, become deeply stained. Bowman, Winternitz and Evans<sup>46</sup> found that trypan blue injected intravenously stained tubercles in experimental tuberculosis. Subsequently Winternitz and Hirschfelder<sup>47</sup> demonstrated that this dye injected in experimental lobar pneumonia stained the consolidated area of the lung selectively: "The intravenous injection of trypan blue and trypan red gave rise to the usual diffuse staining as described by Bouffard, Goldman, etc., but in addition to this the diseased area of the lung showed a much more intense staining than any of the other tissues, while the normal lung tissue was practically normal in color." Lewis<sup>48</sup> found that when the cornea of a rabbit was inoculated with a living culture of the tubercle bacillus, a progressive lesion resulted characterized by intense congestion of the conjunctiva. When the animal received an intravenous injection of trypan red twenty-four hours or more after such an inoculation, the fluid in the anterior chamber of the inoculated eye became colored. Precisely similar results were obtained when abrin was administered in the conjunctiva as an inflammatory irritant. McClellan and Goodpasture<sup>49</sup> showed that trypan blue accumulated in lesions of herpetic encephalitis in the rabbit's brain, the injured areas presenting a striking color against the unstained healthy tissue of the brain. Siengalewicz<sup>50</sup> found that general damage to the

45. MacCurdy, I. T., and Evans, H. M.: *Berl. klin. Wchnschr.* **49**:1695, 1912.

46. Bowman, F. B.; Winternitz, M. C., and Evans, H. M.: *Centralbl. Bakteriologie*. **65**:403, 1912.

47. Winternitz, M. C., and Hirschfelder, A. D.: *J. Exper. Med.* **17**:657, 1913.

48. Lewis, P. A.: *J. Exper. Med.* **23**:669, 1916.

49. McClellan, R. H., and Goodpasture, E. W.: *J. M. Research* **44**:201, 1923.

50. Siengalewicz, S. S.: *J. Pharmacol. & Exper. Therap.* **24**:289, 1925.



nervous tissue, such as poisoning with carbon monoxide or with arsphenamine, was followed by marked staining of the damaged areas by trypan blue. Ramsdell<sup>51</sup> injected trypan blue into the veins of rabbits and guinea-pigs previously treated with foreign serum, and found that injection of the same serum into the skin of the ear immediately caused local infiltration of the dye into the adjacent tissue. She regarded the infiltration by the dye as an indicator of edematous changes resulting from toxic injury to the capillary endothelium.

Okuneff<sup>52</sup> found that a thermal irritant favored the passage of vital stains from the blood stream into the area heated. Kusnetzowsky<sup>53</sup> also observed that the local application to the skin of an irritant such as heat or mustard oil caused an accumulation of trypan blue in the inflamed area when the dye had previously been injected into the blood stream.

I<sup>19</sup> found that trypan blue introduced into the circulating blood rapidly entered the site of inflammation, staining the tissue deeply, and that it did not readily drain away through the tributary lymphatic vessels (fig. 1). I further observed that the longer the interval of time between the injection of the irritant and that of the dye, the more complete was its retention, less of the dye diffusing to the regional lymph node. Thus there was not only a rapid accumulation, but also a fixation of dye from the blood stream in the inflamed area.

It is well known that the retrosternal lymph nodes of the anterior mediastinum drain the peritoneal cavity. When trypan blue was injected into a normal peritoneal cavity the retrosternal lymph nodes were within a short time deeply stained by the dye, but when the dye was injected into a peritoneal cavity in which an inflammatory reaction had been set up by a sterile irritant, such as aleuronat, the dye was wholly or partly prevented from reaching the retrosternal lymphatic nodes. From these studies the following principle has consequently been established: A vital dye, trypan blue, injected into the circulating blood stream rapidly accumulates in an inflamed area and is fixed there, so that it fails to appear in the tributary lymphatics draining the area.

The accumulation in inflamed tissue of the dye that has been introduced into the blood stream is doubtless the result of the increased permeability of the capillaries which is part of the inflammatory reaction.<sup>54</sup> We were able to secure quantitative data by studying directly the change in the concentration of the dye in the blood stream both in the inflamed, and in the normal, mesentery of the frog. After intraventricular injection of the dye the change in concentration of trypan blue within the

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51. Ramsdell, S. G.: *J. Immunol.* **15**:305, 1928.

52. Okuneff, N.: *Arch. f. d. ges. Physiol.* **204**:261, 1924.

53. Kusnetzowsky, N.: *Ztschr. f. d. ges. exper. Med.* **44**:646, 1925.

54. Menkin, V., and Menkin, M. F.: *J. Exper. Med.* **51**:285, 1930.



Fig. 1.—The accumulation of trypan blue at a site of inflammation when the dye was injected into the circulating blood stream. The area of inflammation, which was of four hours' duration, was induced in the skin of the abdomen of a rabbit by injection of concentrated broth. The dye was injected intravenously fifteen minutes after the injection of the irritant.



Figure 2



Figure 3

Fig. 2.—Fixation of injected iron by an inflammatory reaction. Ferric chloride was injected into the normal peritoneal cavity of a rabbit. One hour later the rabbit was killed. The substernal region revealed the presence of iron in the retrosternal lymphatics that drain the peritoneal cavity (prussian blue reaction).

Fig. 3.—Ferric chloride was injected into a peritoneal cavity that had been inflamed for twenty-two hours and forty minutes. An hour later the rabbit was killed. The substernal region failed to reveal the presence of iron to any appreciable extent in the lymphatics when the qualitative test was applied to the tissues.



capillaries could be estimated by a colorimetric method. When the logarithms of the concentrations of the dye within the capillary were plotted against time, a straight line was obtained, denoting an exponential type of relationship. The equations derived by the method of least squares for both the normal and the inflamed areas were of the type:

$$y = be^{-ax}$$

The actual equations found were:

$$y = 7.63e^{-0.24x} \dots \dots (1) \text{ in the normal peritoneal cavity}$$

$$y = 6.9e^{-0.42x} \dots \dots (2) \text{ in the inflamed peritoneal cavity}$$

In these equations  $y$  represents the concentration of dye in the capillaries as estimated by comparison with the nearest standard on the colorimetric scale, and  $x$  stands for the time in minutes after the intraventricular injection of the dye, and  $a$  represents the slope of the curve. In the equation for the control experiments,  $a$  equaled 0.24, while in the equation for the concentration of dye in the capillaries of the inflamed peritoneal cavity,  $a$  equaled 0.42. Since  $a$  is an index of the slope and consequently of the rate of the change of concentration of the dye, it is clear that the rate of the fall of the concentration of trypan blue was almost twice as rapid in the capillaries of the inflamed area as in the capillaries of the normal mesentery. The dye obviously diffused outward into the extracapillary spaces, as could be seen by direct observation and of course by the fact that the cells of the extracapillary spaces were stained. This does not necessarily mean that the fall of the concentration of dye within the capillary is an exact measure of the amount of dye that passes through its walls, for it is conceivable that there may be other factors involved. However, in view of previous studies<sup>19</sup> showing that trypan blue injected intravenously rapidly passes into an inflamed area, it is believed that the increased rate of the fall of concentration is a measure of the increased passage of the dye through the wall of the capillary.

Landis showed that capillaries injured by alcohol and mercuric chloride are permeable to the plasma colloids and approximately seven times more permeable to fluids than the normal capillaries. It seems probable that similarly the inflammatory irritant may have a direct toxic effect on the walls of the capillaries and, by increasing their permeability, may cause a fall in the osmotic pressure of the plasma colloids. This direct injury would result in an increased rate of filtration of the dye into the inflamed area and would account for the increased rate of the fall in the concentration of the dye within the capillaries. Landis,<sup>55</sup> however, recently demonstrated that when a wheal is formed on the skin by freezing, or when a blister is caused by the application of a cantharidal plaster, the average capillary pressure at the point of injury rises appre-

55. Landis, E. M.: *Heart* 15:209, 1930.

ciably. This factor in addition to increased permeability of capillaries also increases the filtration of substances into the extracapillary spaces.

In order to determine whether the accumulation of trypan blue from the circulating blood stream into an inflamed area is due to direct damage to the wall of the capillaries, by the contact of the irritant, the following experiment, suggested by Prof. S. B. Wolbach, was performed. A small area at the center of the cornea of an anesthetized rabbit was burned by the application of a red hot platinum wire. On the following day only the small central area of the injury in the cornea appeared cloudy. At the periphery, however, the tissues overlying the sclera were definitely congested and thickened. Ten cubic centimeters of 1 per cent trypan blue was injected intravenously. Within a very short time the tissues at the periphery of the cornea were deeply stained by the dye. The corresponding normal area in the other eye showed either no presence of the dye or only a minute trace of it. This experiment indicated that the capillaries at the site of inflamed tissue are more permeable to trypan blue than those in normal areas.

Recently Underhill and his collaborators<sup>56</sup> showed that whereas trypan blue injected intravenously rapidly permeates into the edematous part of an area of burned skin, the reabsorption of the dye from this area is a very slow process. On the basis of their observations these investigators concluded that increased permeability of the capillaries in one direction may exist simultaneously with decreased permeability in the opposite direction. The experimental evidence that I<sup>57</sup> presented, although not disposing of their contention, renders such an inference perhaps unnecessary. In the studies of lymphatics draining an inflamed area it was readily demonstrated that the failure of the dye to be reabsorbed from such an area was due to its fixation in situ by the presence of a fine network of fibrin and of thrombosed lymphatics.

Further studies were undertaken to determine whether a metal would, like the dye, be fixed in situ by the inflammatory reaction.<sup>58</sup> Iron was selected because of the ease of detecting this metal in tissues qualitatively by the prussian blue reaction. When colloidal iron or ferric chloride was injected into the normal peritoneal cavities of rabbits, it rapidly accumulated in the retrosternal lymph nodes, as was shown by the prussian blue reaction, but it failed to reach these lymph nodes when it was injected into peritoneal cavities in which inflammation had been caused previously either by aleuronat or by *Staph. aureus* (figs. 2 and 3). Quantitative studies by the method of Kennedy<sup>59</sup> of the iron con-

56. Underhill, F. P.; Kapsinow, R., and Fisk, M. E.: *Am. J. Physiol.* **95**:315, 1930.

57. Menkin (footnotes 54 and 19).

58. Menkin, V.: *J. Exper. Med.* **51**:879, 1930.

59. Kennedy, R. P.: *J. Biol. Chem.* **74**:385, 1927.

tent of these lymph nodes in animals into which ferric chloride had been injected intraperitoneally revealed 56.7 per cent more metal in the nodes of the animals with normal peritoneal cavities than in those with inflamed peritoneal cavities. Experiments were then performed to demonstrate the accumulation of the metal in inflamed areas when ferric chloride was injected into the circulating blood stream. Acute inflammatory reactions of from six to seven hours' duration were obtained by the injection of *Staph. aureus* into the skin of the abdomens of rabbits. Such acute inflamed dermal areas did not themselves give the prussian blue reaction, but when ferric chloride was injected intravenously the metal became demonstrable in these areas by qualitative tests. Quantitative determinations showed in inflamed areas of the skin of normal animals, as an average figure, 9.7 mg. of iron per hundred grams of dry tissue, as compared with 16.2 mg. in animals into which ferric chloride had been injected intravenously, or an increase of 67 per cent in iron content after the introduction of the ferric salt. The average iron content of normal areas of the skin in the animals receiving the injections was 10.4 mg., as compared with 8.4 mg. in the animals not given injections, showing an increase of 23.8 per cent as a result of the injection of the metal salt. Thus about three times more metal accumulated in the inflamed, than in the normal, areas of the skin. These observations show that iron accumulates, as does trypan blue, in an inflamed area when injected intravenously and is fixed there by the inflammatory reaction.

These studies may have a clinical application. It is conceivable that by the accumulation of dye, iron or other material in an inflamed area, the character or the course of development of the inflammatory reaction may be altered. I<sup>60</sup> demonstrated by qualitative and subsequently by quantitative methods that daily intravenous injections of ferric chloride in tuberculous rabbits caused an accumulation of iron in the caseous areas of the tuberculous foci.<sup>60</sup> Whereas in inflamed areas previously studied the iron salt had been found to accumulate at the periphery and not in the central portion (where the circulation is relatively inactive), these tuberculous foci, on the contrary, revealed the metal in the caseous centers. It is to be noted in this connection that various dyes that cannot penetrate living cells are able to stain dead or dying cells.<sup>61</sup> Further experiments are being conducted to determine the effect of the accumulation of iron in tuberculous areas on the course of development of the disease.

As mentioned, Opie<sup>35</sup> showed that foreign protein injected into the skin of an actively immunized animal is fixed at the site of injection,

60. Menkin, V.: Proc. Soc. Exper. Biol. & Med. **27**:1020, 1930. Menkin, V., and Menkin, M. F.: J. Exper. Med. **53**:919, 1931.

61. Stechemacher, S.: Beitr. z. path. Anat. u. z. allg. Path. **57**:314, 1914.

where the contact of antigen and antibody causes an acute inflammatory reaction (Arthus' phenomenon). In view of this work on immunized animals and of the results obtained with trypan blue and iron, I<sup>29</sup> undertook experiments to determine whether a readily identified foreign protein, such as horse serum, injected into an area of inflammation caused by either bacteria or a sterile irritant would also be retained in situ by the inflammatory reaction.

Horse serum was injected into the peritoneal cavities of normal rabbits and of rabbits that had previously been given an intraperitoneal injection of an inflammatory irritant. The presence of the foreign protein was tested by the precipitin reaction on blood samples removed at varying intervals from the heart. It was found that horse serum injected into an inflamed peritoneal cavity penetrated into the blood stream less rapidly than when introduced into the normal cavity. Furthermore it was noted that when the foreign protein was injected into a cutaneous inflammatory area it was held in situ for a longer period than when injected into an inflamed peritoneal cavity.

These experiments showed that, like trypan blue and iron, complex foreign proteins, such as are found in horse serum, when injected into an inflamed area (caused in Opie's experiments by the contact of antigen and antibody and in the present experiments by a sterile irritant) are held fixed by the inflammatory reaction.

With the demonstration that the penetration of horse serum into the blood stream is delayed at the site of inflammation, the attempt was made to determine whether, as with trypan blue and ferric chloride previously studied, horse serum injected into the circulating blood stream would accumulate in inflamed areas to a greater extent than in normal tissue.

Rabbits were used. An area of cutaneous inflammation was induced by the injection of about 0.2 cc. of a saline suspension of *Staph. aureus* into the skin of the abdomen. About three hours later 10 cc. of horse serum was injected intravenously. The animal was killed when the inflammation was of from five to six hours' duration. Saline extracts obtained from the inflamed area and from an area of normal skin were tested for the presence of horse serum. It was consistently found that a greater concentration of the foreign protein could be recovered from the site of inflammation than from the corresponding area of normal skin. These results were therefore similar to those obtained with trypan blue and with ferric chloride. The accumulation of foreign protein in inflamed tissue is doubtless in part the result of the increased passage of fluid from the circulating blood stream, but the observations recorded indicate that its escape from the site of inflammation is retarded.

These observations offered a means of explaining an interesting observation made some years ago by Auer.<sup>62</sup> This investigator showed that when the ear of a rabbit immunized with horse serum was painted with xylene, following reinjection of the homologous antigen into the peritoneal cavity, an intense inflammatory reaction, followed by necrosis, took place in the affected ear. No such severe reaction was seen when xylene had been applied to the ear of a normal rabbit after a single injection of horse serum. In view of the observations on the accumulation of foreign protein in inflamed areas it is highly probable that the reaction in Auer's experiment was simply the result of an accumulation in the inflamed ear of the sensitized animal of antigen and antibody from the circulating blood stream. The contact of antigen and antibody in the tissues evidently caused an acute inflammatory reaction, thus intensifying the mild reaction produced by xylene alone. This explanation is verified by the demonstration in several experiments that horse serum from the circulating blood accumulates in greater concentration in the inflamed, than in the normal, ear of a sensitized animal.

It is also possible that the phenomenon of focal reaction in tuberculosis may be explained by these observations. When tuberculin is introduced into the blood stream of an animal with a tuberculous lesion, an intense inflammatory reaction may develop in the lesion. The mechanism of this focal reaction in tuberculosis is not understood. It is conceivable in view of the aforementioned experiments with horse serum that in a like manner tuberculoprotein from the blood stream may accumulate in the inflamed tuberculous lesion and by its presence there induce a local inflammatory reaction.

Shwartzman<sup>63</sup> described an interesting reaction that occurred when a filtrate of *B. typhosus* was injected into the skin of a rabbit. When twenty-four hours later the same filtrate or a filtrate of a nonrelated organism, such as the meningococcus, was introduced into the blood stream, hemorrhagic necrosis often took place at the site of the cutaneous injection. The observations on the accumulation and fixation of foreign proteins at the site of inflammation which have been described suggest that the intense cutaneous reaction following the intravenous injection of a bacterial filtrate may be the result of an accumulation of this substance in an area of the skin already inflamed, with resulting accentuation of the original lesion. The reaction does not have to take place with all bacterial filtrates or with all inflammatory irritants. The degree of permeability of the capillaries and the optimum of the synergistic action of two irritating substances on one another may modify the final reaction. On conceiving the phenomenon from this angle, it is

62. Auer, J.: J. Exper. Med. **32**:427, 1920.

63. Shwartzman, G.: J. Exper. Med. **48**:247, 1928.



suggested that it may be purely a nonspecific reaction resulting from the accumulation of an irritating substance in an inflamed area. Frisch<sup>63a</sup> recently showed that this reaction took place in the prepared area of the skin if the bacterial filtrate was injected intraperitoneally. When, however, the filtrate had been previously injected several times into the peritoneal cavity, the skin reaction failed to occur when the filtrate was injected intraperitoneally, but not when injected intravenously. The failure of the reaction may perhaps be explained in terms of fixation of the bacterial filtrate in a peritoneal cavity inflamed by preliminary injections of the filtrate.

Further studies were undertaken to determine the behavior of particulate matter and of bacteria at the site of inflammation when injected intravenously or directly into an inflamed area.<sup>15</sup> It was believed that by such studies additional valuable information might be obtained concerning the increased permeability of the capillaries in inflammation and the consequent localization of bacteria from the circulating blood stream when reaching a point of injury. Previous investigators had studied the dissemination of bacteria from the site of inflammation by recovering the organisms in the blood stream. The local state had not been studied. In experiments that I performed it was shown that the failure of bacterial dissemination from the site of inflammation was due to fixation of the micro-organisms *in situ*. It was found that diluted india ink or particles of graphite injected into a normal peritoneal cavity readily drained into the retrosternal lymph nodes. When, however, these particles were injected into an inflamed peritoneal cavity, they failed to disseminate to any extent to the tributary lymphatic nodes.

The attempt was next made to determine whether india ink injected into the circulating blood would rapidly enter the inflamed area. No satisfactory evidence to that effect could be obtained. It is well known in this connection that india ink adheres to the endothelial lining of capillaries. Kusnetzowsky,<sup>64</sup> however, reported that as the vessels become more permeable in areas of inflammation particles of india ink make their way through the endothelial wall into the extracapillary spaces, where they are taken up by the polyblasts. Graphite ink (Hydrokollag "300") has the advantage over india ink of not adhering to the vascular wall.<sup>65</sup> For this reason experiments were performed to determine whether such particles accumulate in an inflamed area when injected into the circulating blood stream. Rabbits were used. An inflammatory reaction was induced in the skin of the abdomen by any one of a variety of sterile or bacterial irritants. Several hours later 10 cc. of a

63a. Frisch, J. A.: *Arch. Int. Med.* **46**:361, 1930.

64. Kusnetzowsky, N.: *Beitr. z. path. Anat. u. z. allg. Path.* **83**:649, 1929.

65. Drinker, C. K., and Churchill, E. D.: *Proc. Roy. Soc., London s. B.* **101**: 462, 1927

diluted suspension of particles of graphite was injected into the aural vein. After a variable interval of time the area of inflammation revealed large deposits of particles of graphite. Histologic section of such an area showed many polymorphonuclear leukocytes loaded with graphite. The question arose as to whether particles of graphite injected into the circulating blood are brought to the site of inflammation entirely by leukocytes or whether the permeability of the capillary is also sufficiently increased to allow some of these relatively large particles to pass through the endothelial wall. To settle this point histologic examination was made of areas of skin in which the duration of inflammation was only of about six hours. Careful studies under oil immersion magnification revealed many capillaries with unattached particles of graphite within their lumina. Some of these particles were in the process of passing into the extracapillary spaces. In the tissue spaces many of these isolated granules were found surrounding the capillaries. Only occasionally in such regions could the particles be seen within polymorphonuclear leukocytes. It is evident, therefore, that when the inflammatory reaction is of short duration the passage of nonphagocytosed particles of graphite through the walls of capillaries can be readily demonstrated. Since no such evidence was obtained in sections of normal areas, the observations indicate that with inflammation the walls of the capillaries become permeable to particulate matter.

Experiments were devised to determine whether bacteria would be fixed like particles of carbon by the inflammatory reaction. The organism studied was *B. prodigiosus*. In a few experiments *B. pyocyaneus* was used. The organisms were injected into both the normal and the inflamed peritoneal cavities of rabbits. After a variable interval of time the retrosternal lymph nodes were removed, and the number of bacilli was determined by a culture method. It was found that whereas a large number of bacilli were recovered from the nodes in the normal animal, a relatively insignificant number were obtained from those of rabbits having inflamed peritoneal cavities.

Experiments were set up to determine whether or not this failure of bacterial dissemination to the tributary lymph nodes was actually due to retention of the micro-organisms at the site of inflammation. In all experiments a larger number of micro-organisms were recovered from the site of inflammation than from a corresponding normal area. It is to be noted that precisely similar results had been obtained with a foreign protein.<sup>29</sup> Rich and McCordock<sup>27</sup> questioned the view that acute inflammation per se can prevent the spread of bacteria. These observations represent direct evidence that bacteria are retained in situ by the inflammatory reaction, and consequently fail to disseminate to the tributary lymphatic nodes as readily as under ordinary circumstances.

It is known that injury to tissue may determine the localization in the damaged tissue of bacteria or of ultrafiltrable organisms present in the circulating blood. Calmette and Guérin <sup>66</sup> found that after the back of a rabbit had been shaved, intravenous injection of the virus of vaccinia resulted in a localization of the virus in the epilated area. Kettle <sup>67</sup> showed that tubercle bacilli injected intravenously into mice or rabbits tended to settle in localized subcutaneous lesions caused by various agents that produce increased vascularity and necrosis of tissue. Chesney, Turner and Halley <sup>68</sup> found that when rabbits with wounds in their backs were inoculated either intratesticularly or intravenously with *Spirochaeta pallida*, syphilitic lesions invariably developed in the wounds. Findlay <sup>69</sup> believed that histamine-like substances, liberated at the point of injury, were the specific agents, which by their action on the walls of the capillaries caused organisms present in the blood stream to localize at the site of injury. Sager and Nickel <sup>70</sup> recently demonstrated that subcutaneous injection of silver nitrate into rabbits caused sterile abscesses. After intravenous injection of green streptococci, these organisms were recovered from the abscesses in some of the rabbits. They concluded that bacteria may become localized in places of lowered resistance. Quednau <sup>71</sup> pointed out that bacteria, especially pneumococci, in the blood stream tended to localize readily in areas of cerebral softening.

I undertook experiments to determine whether bacteria which, when injected directly into an inflamed area, are fixed in situ would accumulate rapidly at the site of inflammation when injected into the blood stream.<sup>15</sup> Rabbits were used. An area of inflammation was induced by injecting 0.5 cc. of concentrated broth in the skin of the abdomen. After a short time a suspension of *B. prodigiosus* or of *B. pyocyaneus* was injected intravenously. When a variable interval of time had elapsed, the animal was killed and the number of micro-organisms localized in both the inflamed and a normal area of the skin was determined by culture of the respective tissues. The number of intravenously injected bacteria accumulating at the site of inflammation was found to be distinctly greater than that in an area of normal skin. These observations are in agreement with the results described in which it was demonstrated that

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66. Calmette, A., and Guérin, C.: Ann. Inst. Pasteur **15**:161, 1901.

67. Kettle, E. H.: Brit. J. Exper. Path. **5**:158, 1924.

68. Chesney, A. M.; Turner, T. B., and Halley, C. R. L.: Bull. Johns Hopkins Hosp. **42**:319, 1928.

69. Findlay, G. M.: J. Path. & Bact. **31**:633, 1928.

70. Sager, W. W., and Nickel, A. C.: Arch. Surg. **19**:1086, 1929.

71. Quednau, F.: Beitr. z. path. Anat. u. z. allg. Path. **83**:471, 1929.

the permeability of the capillaries in inflamed areas is sufficiently increased to allow particles of carbon to pass through the endothelial wall.

The frequent localization of bacteria from the blood stream in a "*locus minoris resistentiae*" occurring after preliminary trauma or other injury of tissue is well known to pathologists and clinicians. My observations<sup>15</sup> on the accumulation of bacteria in an inflamed area from the circulating blood stream may explain the mechanism of this phenomenon in terms of increased permeability of the capillaries, with resulting accumulation and fixation of bacteria at the point of injury.

#### THE MECHANISM OF FIXATION BY THE INFLAMMATORY REACTION

With the demonstration that the accumulation of foreign substances from the circulating blood in an inflamed area is associated with increased permeability of the capillaries, studies were extended in an attempt to determine the mechanism of the fixation by the inflammatory reaction.<sup>4a</sup>

The leukocytes are probably not a very significant factor in the mechanism of fixation, for two reasons. In the first place, no definite histologic evidence could be obtained of phagocytosed particles in the leukocytes of the inflamed area at a time when fixation of foreign substances was demonstrable by examination of the tributary lymphatics. In the second place, fixation of trypan blue at the site of inflammation occurred as early as thirty minutes after the injection of the inflammatory irritant.<sup>19</sup> The occurrence of fixation at this early stage of the inflammatory reaction when there were as yet relatively few leukocytes present seems to point toward some other agent responsible for fixation.

A factor that might explain fixation is mechanical obstruction. It is conceivable that a network of fibrin and thrombosed lymphatics at the site of inflammation might arrest the passage of particulate material injected into the inflamed area. The dissemination of fluids would probably also be retarded by mechanical obstruction of this kind, though probably not as effectively as that of solid particles, which would be more readily caught in a fibrinous network. In this connection it is interesting to note that some years ago Opie<sup>72</sup> showed that when cantharidin was administered intramuscularly, the flow of lymph in the thoracic duct was at first diminished, but later might be increased. The decrease in the flow of lymph was accompanied by acute edema of the liver and gallbladder. This edema was due to a plugging of the afferent

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72. Opie, E. L.: J. Exper. Med. **16**:831, 1912.

lymphatic vessels and the sinuses of lymph nodes that drain these organs by fibrin. The observations of Adami mentioned at the beginning of this review are also significant in this connection.

In the endeavor to throw some light on the mechanism involved in fixation, a series of experiments was undertaken to determine whether the inflammatory exudate in itself possesses some property that might facilitate the fixing of foreign substances in the inflamed area.

It was found<sup>73</sup> that when ferric chloride was added directly to the inflammatory exudate heavy precipitation occurred. The compound formed was presumably a ferric proteinate.<sup>74</sup> When horse serum was added to the exudate and incubated at 37 C. for a short time, coagulation took place. However, when trypan blue was added to an inflammatory exudate, no effect was noticed. Yet this vital dye was shown to be definitely held in situ by the inflammatory reaction. The reaction of fixation must be due primarily to some mechanism other than precipitation or coagulation of foreign substances by the inflammatory exudate. As it was shown, however, that iron compounds are apparently more effectively held by the inflammatory reaction than trypan blue, it is possible that precipitation or coagulation of foreign substances acts as a secondary factor in the mechanism of fixation by preventing rapid dissemination from the site of inflammation.

Sections were made of the inflamed tissues of rabbits in experiments in which either trypan blue or ferric chloride had been shown to be fixed in situ by the inflammatory process. In these sections there is shown, as a rule, a central area of dense leukocytic infiltration. The intensity of the inflammatory reaction adjacent to veins and arteries is noteworthy. It is to be recalled<sup>19</sup> that when the dye was injected intravenously, it would not always penetrate into the central zone of the inflamed area. This was evidently due to thrombosis of the small blood vessels, for sections of such areas reveal some thrombosed vessels with acute inflammatory changes in the surrounding tissue.

Histologically there is little evidence of phagocytosed particles of trypan blue or of iron within the leukocytes at a time when retention of these substances at the site of inflammation is clearly demonstrable.

A mesh of fibrin is usually found at the periphery of the zone of dense infiltration (fig. 4). In the same region careful study reveals many lymphatic vessels that are thrombosed; one of these is illustrated in figure 5. The thrombus is characterized by numerous leukocytes within a delicate fibrinous reticulum. The fact that there are many occluded lymphatics and a dense network of fibrinous strands within tissues that are distended with edema at the site of inflammation sup-

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73. Menkin (footnote 72, first reference).

74. Smythe, C. V., and Schmidt, C. L. A.: *J. Biol. Chem.* **88**:241, 1930.



Fig. 4.—The mesh of fibrin at the periphery of a zone of infiltration.

ports the view that foreign substances, especially solid particles, such as precipitated iron salts, can disseminate only with difficulty from the inflamed area through the regional lymphatic vessels.

If, as described, the thrombosed lymphatics and the network of fibrin in the acutely inflamed area are instrumental in mechanically preventing the free passage of substances from the site of inflammation, it

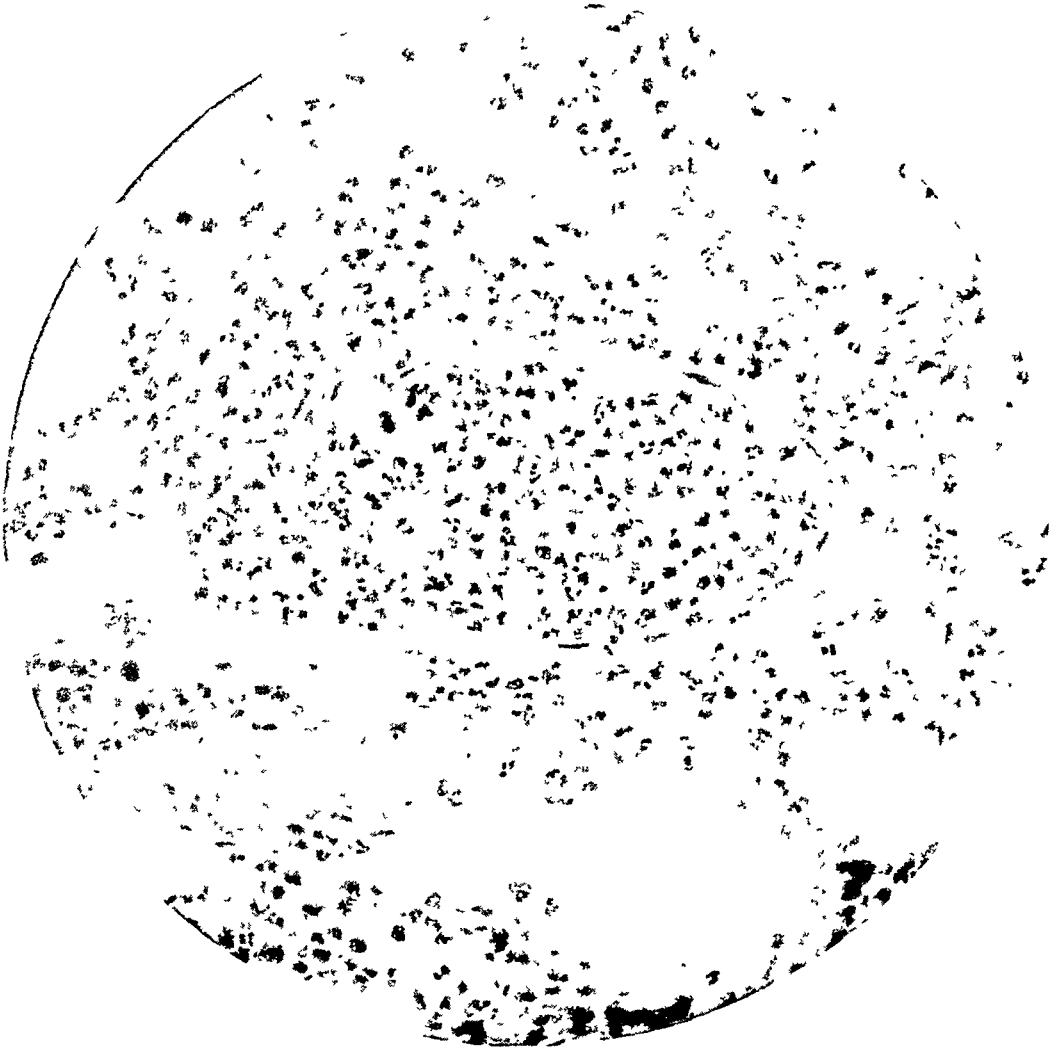


Fig. 5.—A thrombosed lymphatic vessel in a zone of infiltration (from the *Journal of Experimental Medicine*).

follows that similar substances injected at the periphery of the inflamed area should be prevented by the same obstruction from entering it. To test this hypothesis, the following experiments were conducted at the suggestion of Professor Opie.

An inflammatory reaction was induced in the skin of the abdomen of a rabbit by the use of a bacterial irritant (*Staph. aureus*) or by the injection of concentrated broth. After a varying interval of time trypan



Fig. 6.—The failure of trypan blue to penetrate a site of inflammation when injected at its periphery: 0.1 cc. of trypan blue was injected into each of several areas surrounding an inflamed area. About one and a half hours later the dye had diffused into the surrounding normal tissues, but had failed to penetrate the site of inflammation.





blue was injected at the periphery of the inflamed area in from four to six places. In this way the inflamed area became circumscribed by a blue band. In an area of normal skin similar injections of dye were made to serve as control. Several hours later the inflamed area within the original circumscribed blue band showed no trace of dye, whereas the area of normal skin was diffusely blue (fig. 6). The dye had evidently failed to penetrate into the site of inflammation, when injected at its periphery, owing to the presence of thrombosed lymphatics and of a fine network of fibrin in the tissue spaces. Experiments of the same type repeated on frogs yielded similar results.

Direct observation with a binocular dissecting microscope demonstrated<sup>75</sup> that in frogs the failure of the dye to penetrate into an inflamed area was due to the mechanical obstruction of the lymphatic vessels by blood clots; histologic sections revealed a network of fibrin in the tissue spaces of the inflamed areas.

Similar results were obtained with bacteria.<sup>15</sup> When *B. prodigiosus* was injected at the periphery of an inflamed area the micro-organisms failed to penetrate to any appreciable extent into the site of inflammation; but they disseminated rapidly into normal tissue.

Fixation of foreign substances by the inflammatory reaction is therefore primarily due to mechanical obstruction caused by a fine network of fibrin and by thrombosed lymphatics at the site of inflammation. Further experiments are being conducted to determine the relation between exudation from blood vessels and change in the flow of lymph from the site of inflammation.

The experiments described present proof that various foreign substances, including a dye, a metallic salt, a foreign protein, particulate matter and bacteria, injected into the site of inflammation are fixed in situ and fail to drain readily into the tributary lymphatic vessels. These same substances injected intravenously accumulate rapidly in inflamed areas. This accumulation is partly associated with increased permeability of the capillaries, but is also the result of the inability of these substances to escape from the site of inflammation, owing to the presence of a fine network of fibrin and of thrombosed lymphatics. The mechanism of fixation takes place extremely early in the inflammatory process. In previous experiments<sup>19</sup> fixation of a dye at the site of inflammation could be demonstrated by study of the regional lymphatics as early as thirty minutes after the injection of the inflammatory irritant. This would indicate that the earliest change in inflammation is an increase in the permeability of the capillaries which permits the passage of fibrinogen from the plasma into the tissue spaces. The rapid formation of a network of fibrin and of thrombi in lymphatics at the site of inflam-

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75. Menkin (footnote 4a., second reference).

mation circumscribes the irritating substance and thus prevents its passage into the blood stream. This allows a definite interval of time for the leukocytes to assemble for phagocytosis. The initial fixation of bacteria or of other injurious substances at the site of inflammation thus becomes a protective mechanism and plays a definite rôle in immunity.

Dr. S. B. Wolbach supplied the photomicrographs and Miss E. Piotti the colored plates.

## Notes and News

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**University News, Appointments, Promotions, Resignations, Deaths, etc.**—R. E. Miller has been appointed assistant professor of pathology in the Dartmouth Medical School, Hanover, N. H.

E. B. McKinley, professor of bacteriology in Columbia University and director of the Porto Rico School of Tropical Medicine, has been appointed dean of the school of medicine of the George Washington University, Washington, D. C.

James R. Cash, formerly professor of pathology in Peiping Union Medical College, now occupies the Walter Reed professorship of pathology in the University of Virginia, vacated by the death of Harry T. Marshall.

D. H. Bergey, formerly professor of hygiene and bacteriology in the University of Pennsylvania, has been appointed director of research and biology by the National Drug Company.

J. C. Geiger, professor of epidemiology in the University of California, has been appointed health officer of the city and county of San Francisco.

LaVerne A. Barnes, senior instructor in bacteriology in the school of medicine of Western Reserve University, has resigned to become immunologist to the state department of health of Massachusetts.

Newell R. Ziegler has been appointed associate professor of bacteriology in the University of Missouri.

J. F. Rinehart has been appointed assistant professor of pathology in the University of California.

H. E. Teasley has been appointed instructor in bacteriology in the University of Colorado.

William Freeman has been appointed pathologist at the Worcester State Hospital, Worcester, Mass.

It is reported that Carl V. Weller has been appointed professor of pathology and director of the pathological laboratory at the University of Michigan to succeed the late Aldred S. Warthin.

The Baly Medal of the Royal College of Physicians of London, conferred alternate years on the person deemed to have distinguished himself most in the science of physiology during the two immediately preceding years, has been awarded to W. B. Cannon, professor of physiology in the Harvard Medical School.

The three hundred thousandth microscope made by the firm of E. Leitz has been presented to Prof. Ludwig Aschoff, Freiburg, Germany.

A. F. DeGroat has been appointed assistant professor of pathology in the University of Arkansas.

**Society News.**—During 1932 the Pathological Society of Great Britain and Ireland will hold a meeting at Guy's Hospital in January and at Oxford in the summer.

The German Pathological Society will meet in Rostock during the week of May 14 to 21, 1932, under the presidency of G. Herxheimer. The subjects for special reports are: (1) The Pathologic Anatomy of the Spinal Column, referee, G. Schmoll, and (2) Glioma, referee, Folke Henschen. According to the last report, the society now has 406 members.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

THE BEHAVIOR OF LEAD IN THE ANIMAL ORGANISM. ROBERT A. KEHOE and FREDERICK THAMANN, *Am. J. Hyg.* **13**:478, 1931.

The experiments recorded in this article show that tetra-ethyl lead is absorbed through the skin. The initial distribution of the lead in the tissues in rapid tetra-ethyl lead absorption corresponds to that of an oil-soluble material, and indicates therefore that some portion of the tetra-ethyl lead is absorbed and circulates as such. However, tetra-ethyl lead is rapidly decomposed by the tissues, including the skin, so that only a small portion of the lead found later in the blood is in the form of tetra-ethyl lead. As a net result, after a period of from three to fourteen days, all of the lead in the animal tissues is distributed in a manner characteristic of water-soluble lead compounds. In small dosages, the factors of rapid decomposition and low concentration in the blood so interfere with the distribution of tetra-ethyl lead as such, as to prevent its primary absorption by the nervous system. Even when absorbed as the tera-ethyl compound, the excretion follows quantitatively that of water-soluble lead compounds. Tetra-ethyl lead poisoning is therefore not different from lead poisoning occasioned by other lead compounds. Evidence is adduced showing that the absorption of tetra-ethyl lead from gasoline in concentrations not in excess of 0.1 per cent is inappreciable.

AUTHORS' SUMMARY.

ABSORPTION OF CALCIUM FROM THE GALL BLADDER. EDMUND ANDREWS and LEO HRDINA, *Am. J. M. Sc.* **181**:478, 1931.

In obstruction of the cystic duct in dogs, the relatively high calcium content of the bile of the cystic duct is gradually lowered, and in the presence of infection it is rapidly lowered.

AUTHORS' SUMMARY.

THE RÔLE OF DIET IN PROTECTING AGAINST LIVER INJURY BY ARSPHENAMINE. E. B. CRAVEN, JR., *Bull. Johns Hopkins Hosp.* **48**:131, 1931.

A diet high in fat and one high in protein protect against injury to the liver by arsphenamine, the diet high in fat being the more effective. A diet high in carbohydrate and starvation favor the injurious action of arsphenamine.

PATHOGENESIS OF MULTIPLE SCLEROSIS. RICHARD M. BRICKNER, *Bull. Neurol. Inst., New York* **1**:105, 1931.

In a previous contribution (*Arch. Neurol. & Psychiat.* **23**:715, 1930) Brickner came to the conclusion that the serum of patients suffering from multiple sclerosis contains a substance that destroys the myelin; it causes myelinolysis. The hypothetical substance was supposed to be a lipase. Brickner studied fifty-one patients with multiple sclerosis and seventy-nine normal persons to test this hypothesis. From his laborious and highly technical study, Brickner concludes that the blood of a patient with multiple sclerosis contains an abnormal lipase, and that most likely one does not deal in this disease with a filtrable virus. The lipase is supposed to be the same agent that produces myelinolysis in the spinal cord in rats. Some of the facts established by the present studies are that in multiple sclerosis acid is produced in the blood serum, and that the production of acid can be inhibited by the addition of sodium oxalate. In lecithinated serum, acid is formed from preparations of the serums of both normal persons and patients with multiple sclerosis, but it is greater in the serum of the latter and may be markedly inhibited by oxalate.

GEORGE B. HASSIN.

THE INFLUENCE OF THE INTRAVENOUS INJECTION OF UREA ON THE EXCHANGE OF SUBSTANCES BETWEEN THE BLOOD AND THE TISSUES. HUGH DONOVAN and O. BRENNER, *Brit. J. Exper. Path.* **11**:419, 1930.

The results of the intravenous injection of 5 Gm. of urea in health and in various diseases were investigated. By the time the urea had mixed with the blood (three minutes), 80 per cent had left the blood stream. It is suggested that the urea leaves the blood by a process of simple diffusion, passing into the water of the cells, as well as into the free water, and that the process is complete in fifteen minutes, when the concentration of urea in the blood equals that in the tissues. A review of the literature indicates that urea is unique in this respect. With no other substance investigated is the distribution uniform over blood and tissues, and with most substances, the concentration in some tissues is greater than that in the blood, showing that the process by which the substance enters the tissues is not one of simple diffusion alone. It is suggested that the sudden introduction of urea into the body delays the formation of urea, and may even bring it to a standstill for the first two hours after the injection. The importance of this in connection with the production of uremia is mentioned. The osmotic attraction of the urea injected is greatly reduced by its rapidly leaving the blood. Salt also leaves the blood, so that the osmotic attraction of dissolved substances in the blood is not raised even three minutes after the injection. In spite of this the blood is more dilute at the end of three minutes. It is suggested that this may be due to a lag in the movement of water behind that of urea and salt. Evidence is discussed in favor of the view that, except in edematous patients, injection of urea causes some salt to pass out of the fluid of the body, to be stored in a "dry" form in the salt depots of the body, thus reducing the crystalloid concentration of the fluids of the body, which had been raised by the injection of urea; but that in most cases of edema, salt is liberated from the depots and enters the fluids of the body. It is pointed out that the capillaries are completely permeable to water and to most of the substances dissolved in the blood plasma, and are partly permeable to the plasma proteins. The factors in the retention of the blood inside the vessels are discussed. None of the causes ordinarily mentioned seem sufficient. It seems probable that the true cause must be sought in the tissues, rather than in the blood or in the blood vessels. It is suggested that perhaps the amount of fluid leaving the blood is limited by the capacity of the tissues for taking up water, and that this in its turn may be influenced by changes in the balance between acid and base, or by alterations in the relative proportions of various ions. A review of the literature shows that in experimental edema usually less fluid passes into the tissues, after intravenous injection, than in nonedematous animals, suggesting that an important factor in the production of edema is a deficient power of the tissues to hold and bind water, which therefore accumulates in the tissue spaces. Our own results suggest that in edema the tissues are also less able to bind salt, which also accumulates in the free fluids of the tissues. Any satisfactory theory of edema must account for this.

AUTHORS' SUMMARY.

THE SIGNIFICANCE OF THE LUNGS FOR THE TRANSPORTATION OF MATERIAL WITHIN THE BODY. P. SPANIER, *Beitr. z. Klin. d. Tuberk.* **76**:507, 1931.

The lungs retain a considerable number of leukocytes that have phagocytosed foreign particles. Such particulate matter is finally excreted again, but the roads of transport are not definitely known. Experiments are being made to use this leukocytic carrying mechanism in order to concentrate therapeutic agents in the lung tissue.

MAX PINNER.

THE ACTION OF THE BLOOD OF PREGNANT WOMEN ON FROG LARVAE. H. EUFINGER, H. WIESBADER and N. SMILOVITS, *Klin. Wchnschr.* **10**:348, 1931.

The blood of pregnant women fed to frog larvae influences their metamorphosis in the same way, if not as profoundly as does thyroxin. This effect is correlated with hyperfunction of the thyroid gland during pregnancy.

EDWIN F. HIRSCH.

FUNCTIONAL DISTURBANCES IN THE CENTRAL NERVOUS SYSTEM IN CEREBRAL ANEMIA. J. R. PETROFF, *Ztschr. f. d. ges. exper. Med.* **75**:1, 1931.

Experiments and observations are cited which show that marked disturbances in respiration are accompanied by definite changes in the vasomotor center. Stimulation of the respiratory center in repeated cases of cerebral anemia is accompanied by simultaneous stimulation of the vasomotor center. The vasomotor center is sensitive to changes in blood pressure in the brain. The author's experiments do not confirm the belief that carbon dioxide and substances such as lactic acid stimulate the vasomotor center and immediately release a pressor effect. The pressor action in asphyxia appears to result from stimulation of the respiratory center. Experiments are also described that show the difference between the action of the vasomotor center (vasoconstriction) and other central activity, such as the control of vascular tone.

PEARL ZEEK.

EXPERIMENTAL OBSTRUCTIVE JAUNDICE. B. VARELA FUENTES, J. DUOMARCO and A. MUNILLA, *Ztschr. f. d. ges. exper. Med.* **75**:577, 1931.

Changes in the glycogen metabolism of the liver following obstruction of the common bile duct were studied in dogs. Although the total amount of hepatic glycogen was found to be decreased, the liver was still capable of building and storing glycogen from excessive carbohydrate taken in food. The hyperglycemia following injections of epinephrine in these animals was similar to that in normal dogs, except that the height of the sugar content of the blood was reached after a somewhat longer interval. The curve of glycosuria following the injection of epinephrine was also essentially the same.

PEARL ZEEK.

THE IMPORTANCE OF SPLENOMEGALY IN ESSENTIAL THROMBOPENIA. R. BERGQVIST, *Acta path. et microbiol. Scandinav.* **8**:1, 1931.

Essential thrombopenia is ascribed by some investigators to disturbances in the marrow, and by others to excessive destruction of platelets in the spleen. The latter view seems to be supported by the good effects of removing the spleen in typical cases of the disease. Bergqvist describes the spleen removed in two typical instances: Grossly, the organ was moderately enlarged; microscopically, there was hyperplasia of germinal centers in follicles and pulp cells and the sinus endothelium, with indications of myeloid transformation (oxydase-positive granules). The view is advanced that the spleen somehow is directly concerned in essential thrombopenia, but its precise rôle cannot be defined.

XANTHOMATOSIS WITH CRANIAL DEFECTS (SCHÜLLER'S DISEASE). FREMANN-DAHL and FORSBERG, *Norsk mag. f. lægevidensk.* **92**:523, 1931.

In the form of xanthomatosis called Schüller's disease the xanthomatous infiltrations on the inner side of the cranium lead by pressure atrophy to the destruction of bone. In a girl, aged 14, with a history of trauma against the left temple at the age of 5, attacks of pain in the left temporal region set in three years later, which gained in frequency and intensity, and were followed by symptoms of slowly increasing intracranial pressure, together with diabetes insipidus, exophthalmos (left) and retardation of growth. Roentgen examinations showed the development of numerous sharp defects in the cranium, localized especially in the squamous portion of the temporal bone and also in the anterior part of the skull and left orbit, and revealed a typical maplike skull (Schüller). The diagnosis of xanthomatosis was supported by a negative Wassermann reaction, the presence of Bence-Jones protein in the urine, and by high blood cholesterol, pointing to a disturbance in the lipid metabolism.

## Pathologic Anatomy

MALIGNANT ENDOCARDITIS (PNEUMOCOCCAL), WITH EARLY CALCIFICATION AND CALCAREOUS RENAL EMBOLI. E. R. CULLINAN and W. S. BAXTER, *Am. Heart J.* **6**:420, 1931.

In a case of malignant endocarditis in a youth of 19 years, autopsy twenty days after the onset revealed calcification in the newly formed vegetations of the infected valve. The arterioles of the kidney contained calcified emboli. These had caused, not infarction by occlusion, but hemorrhage by trauma, in the adjacent renal substance.

ALFRED M. GLAZER.

SCLEROSIS OF SMALL MESENTERIC VESSELS WITH ULCERATION AND GANGRENE OF THE ENTERIC TRACT. PEARL ZEEK and JOHN J. PHAIR, *Am. J. M. Sc.* **181**: 548, 1931.

Three cases of extensive ulceration and gangrene of the gastro-enteric tract are described, in which these lesions are attributed to mesenteric arteriosclerosis.

AUTHORS' SUMMARY.

RENAL CALCIFICATION. S. PETRASSI, *Arch. di path. e clin. med.* **10**:104, 1930.

The various forms of renal calcification are classified by the author not only by topographic differences, but also by pathogenesis. Special attention is given to the "metastases of calcium" in the kidney. Calcifications in the kidney were found especially in chronic glomerulonephritis and primary sclerosis. The calcium can be found in the form of stones in cortical cysts, granules in hyalinized glomeruli, deposits in normal glomeruli and deposits in the arterial wall. The author believes that the majority of these calcium deposits are a sign of disturbed elimination of the calcium through the tubular epithelium.

E. HAAM.

EXPERIMENTAL PRODUCTION OF ENDOMETRIOSIS. C. R. FUMAGALLI, *Riv. di pat. sper.* **6**:154, 1931.

The three theories of the origin of endometriosis are discussed. The object in the experiments has been to test the theory of the endometrial origin. The endometrium of an amputated end of the uterus of the dog was transplanted in the ovaries and disseminated over the peritoneum. It was found that the endometrial cells can attach themselves easily and form growths of the general structure seen in human endometriomas.

E. HAAM.

PATHOLOGIC ANATOMY OF TUBERCULOSIS IN CHILDREN. H. KUDLICH, *Beitr. z. Klin. d. Tuberk.* **75**:575, 1930.

In Ghon's institute, in the years from 1926 to 1929, autopsies were performed on 136 children with tuberculosis. In 90 per cent of these cases, the primary infection occurred by way of the lungs. In 2.5 per cent there was a primary intestinal infection. In a girl, 6 years old, a partly calcified subpleural nodule was found, which had the appearance of a primary focus, but no lesion could be found in the regional lymph nodes. Microscopic examination showed that this nodule was not caused by tubercle bacilli, but by an unidentified parasite. Two cases with typical primary foci showed no lymphoglandular component ("rudimentary primary complex"). The localization of the primary foci was in general the same as described by Ghon. About one fourth of all primary foci showed excavation; this was noted in half of all infants. It is emphasized again that all primary pulmonary foci are exudative lesions, and that all evidence favors Ghon's opinion that the causative bacilli reach the alveoli by the respiratory tubules, and not by the blood or by the lymph stream. In 49 of the 136 children with tuberculous lesions, tuberculosis was not the cause of death. Chronic, isolated phthisis (the adult type)



was found in 4 children (ages  $7\frac{1}{2}$  months,  $1\frac{1}{2}$ , 12 and 13 years). Seventy-four of the bodies revealed hematogenous propagation; 54 of these children had died of tuberculous meningitis. Six cases of so-called "endogenous lymphoglandular reinfection" are described in some detail.

MAX PINNER.

APICAL PLEURAL INDURATIONS. C. BOHNE, *Beitr. z. Klin. d. Tuberk.* **76**:164, 1930.

Apical pleural indurations can be differentiated macroscopically into three groups: (1) diffuse adhesive apical pleuritis, (2) localized adhesive pleural scar and (3) cartilage-like induration of the apical pleura. Histologic examinations showed that in the first variety small tuberculous foci are found in the thickened pleura. The subpleural tissue shows usually tuberculous processes, either scarred or active. This type was found in 10.1 per cent of the cases. The second type is usually seen in the area of the apical or subapical bronchiolus. The underlying pulmonary parenchyma usually shows anthracotic scars. These reveal microscopically either a typical Puhl focus or a caseous bronchitis. In the majority of these cases, however, the parenchyma shows only a hyaline scar in which no specific alterations are seen. This type of lesion was seen in 31.8 per cent of the cases. The third type is a hyalinized induration of the visceral pleura without involvement of the parietal pleura. The underlying pulmonary tissue is collapsed and anthracotic. Tuberculous foci are absent. The frequency of nontuberculous pleural indurations is emphasized.

MAX PINNER.

A RARE DIAPHRAGMATIC ANOMALY. W. PUTSCHAR, *Centralbl. f. allg. Path. u. path. Anat.* **50**:97, 1930.

A combination of diaphragmatic fenestration and hernia is reported in the body of a new-born girl. The right half of the diaphragm was normal; the left was partially absent in the anterior part and allowed herniation of the left lobe of the liver, stomach, tail of the pancreas, spleen and large and small bowel into the left pleural cavity, in part covered by thin diaphragm. Instead of the usual arrangement, in which all muscle strands in the diaphragm radiate to the central tendon, the only well developed muscle band in this body ran from the ribs to the vertebral column. Anterior to this band the left leaf of the diaphragm was thin and fenestrated, with muscle bundles occasionally in evidence; dorsal to this band the diaphragm was composed only of fused pleura and peritoneum. In addition to the aforementioned anomalies there were a mobile duodenum, a common mesentery for the small and large bowel and atypical peritoneal bands between the undersurface of the liver and the right kidney, and between the duodenum, cecum and appendix.

GEORGE RUKSTINAT.

FATTY CHANGES IN THE PULMONARY VEINS. R. PRÉVÔT, *Centralbl. f. allg. Path. u. path. Anat.* **50**:305, 1931.

Marked fatty changes in the pulmonary veins were found in eight and moderate fatty changes in thirty of sixty bodies, ranging in age from 4 months to 86 years. The changes were classified as occurring in elastic or nonelastic tissues, and those occurring in elastic tissues were predominant. A possible etiologic relationship was investigated in acute and chronic pulmonary, renal, cardiac and vascular disease. The only disease seeming to bear such a constant relationship was sclerosis of the pulmonary arteries.

GEORGE RUKSTINAT.

A PERICARDIAL DIVERTICULUM. H. P. YPSILANTI, *Centralbl. f. allg. Path. u. path. Anat.* **50**:417, 1931.

A pericardial diverticulum 9 by 3 by 1 cm., communicating with the pericardial sac through an opening 0.5 cm. in diameter, was found 2 cm. below the mouth of the inferior vena cava, in the body of a 34 year old man who had died of pul-

monary tuberculosis. An acute pericarditis was present in both the main portion of the sac and the diverticulum. There were no adhesions to indicate traction as a factor in the production of this condition.

GEORGE RUKSTINAT.

TWO GALLBLADDERS IN ONE PERSON. A. J. STRUKOW, *Centralbl. f. allg. Path. u. path. Anat.* **50**:420, 1931.

Two gallbladders, each 5 cm. long, were found attached to the liver of a 14 months old child who had died of scarlet fever. The cystic duct of the right bladder was 1 cm. long; that of the left, 1.5 cm. long. The openings of these into the common bile duct were 1 cm. apart. The cystic artery divided into a branch for each bladder at the level of its neck. No other abnormalities were found in the body.

GEORGE RUKSTINAT.

FOUR EXTRASELLAR HYPOPHYSEAL TUMORS. E. DEÁK, *Centralbl. f. allg. Path. u. path. Anat.* **51**:1, 1931.

Deák examined four hypophyseal tumors, which varied in size from that of a pigeon's egg to that of a hen's egg. These were highly cellular, the cells resembling the chief variety seen in the hypophysis. Graz, where the aforementioned material was obtained, is notorious for its endemic goiter. The author is of the opinion that a thyroid gland insufficiency might lead to hypophyseal hyperplasia. In only one of the bodies examined was a goiter present.

GEORGE RUKSTINAT.

RUPTURED ANEURYSM OF THE DUCTUS ARTERIOSUS. A. GUGGENHEIM, *Frankfurt. Ztschr. f. Path.* **40**:436, 1930.

A case of ruptured aneurysm is reported in a girl 20 months of age. The aneurysm had ruptured into the lower lobe of the left lung and into the left pleural cavity. In addition, the case revealed an empyema of the left knee joint. Histologically, the ductus arteriosus in the region of the rupture revealed a hemorrhage of the intima in several places. The intima was thickened, and the media was markedly fibrosed. The point of rupture could be demonstrated in an area 2 mm. distant from the aorta. The author states that, on account of the empyema of the knee joint, it is possible that this is a case of mycotic aneurysm, even though the inflammatory changes in the wall of the ductus arteriosus were very slight. Streptococci could be cultivated from the pus of the knee and from the spleen.

O. SAPHIR.

TWO CASES OF CYSTS OF THE PANCREAS. T. REEKE, *Frankfurt. Ztschr. f. Path.* **40**:444, 1930.

The first case reported was one of cystoma of the pancreas. It was found incidentally in a woman 76 years of age; it measured 1.5 by 2.4 cm. Microscopically, several small cysts were found, some of which had fused. The cysts were lined by one layer of flat epithelial cells. There was no evidence of malignancy. The tumor was well encapsulated. The second case was that of a woman of 54 with a cyst of the pancreas, the size of a child's head. The content of the cyst consisted of partly liquid, partly clotted, blood, with much necrotic tissue. The floor of the cyst was formed by necrotic pancreatic tissue. The cyst is interpreted as a pseudocyst, occurring in a pancreas that was the seat of an acute hemorrhagic pancreatitis.

O. SAPHIR.

INTERSTITIAL PNEUMONIA. L. HONECKER, *Frankfurt. Ztschr. f. Path.* **40**:477, 1930.

A case of interstitial pneumonia is reported in a man, aged 59. The diagnosis was lobar pneumonia involving the right lung. At autopsy, 800 cc. of a yellowish fibrinous fluid was found in the right pleural cavity. Formaldehyde was injected

into the lungs before they were opened. On section, the septums were found to be larger than normal and yellow, and they showed some pus, especially in the lower lobe. Microscopically, the septums were broad and contained dense polymorphonuclear leukocytic infiltrations. The lymph spaces were markedly dilated and filled with polymorphonuclear leukocytes. In some portions, the alveoli contained an edematous liquid, polymorphonuclear leukocytes, red blood cells and desquamated cells. There also were several abscesses. Streptococci were found in sections taken from the right lung. The opinion is expressed that streptococci are the specific cause of interstitial pneumonia.

O. SAPHIR.

**MULTIPLE TUBEROUS OSTEOMAS OF THE LUNG.** S. M. DERISCHANOFF, Frankfurt. *Ztschr. f. Path.* **40**:485, 1930.

A case of osteomas of the lung is reported in a man, aged 21. There was also a chronic endocarditis of the mitral valve with insufficiency of the valve and stenosis of its orifice. Multiple emboli were found in the branches of the pulmonary arteries. The lungs revealed many well circumscribed nodules, the size of peas, which could easily be shelled out. They were white and of bony consistency. Histologically, they consisted of typical bone structures with marrow spaces and haversian canaliculi. The author believes that the osteomas were the result of an organized pneumonia from which the patient had suffered four years previously.

O. SAPHIR.

**CONTRACTION OF THE SUPRARENAL GLANDS AND ATROPHY OF THE TESTES.** E. GÜNTZ, Frankfurt. *Ztschr. f. Path.* **40**:490, 1930.

A case of Addison's disease is described. It presented a clinical picture of a paratyphoid infection, and at autopsy showed a cytotoxic contraction of the suprarenal glands. It also showed a marked atrophy of the testes in which the interstitial cells were markedly decreased. The attempt is made to correlate the changes in the suprarenal cortex with those in the testes. The author believes that the atrophy of the testes followed the changes in the suprarenal cortex. It is possible that the hypophysis, which was not examined histologically, played a contributory rôle in causing the atrophy of the testes. As an example of a relationship between changes in the endocrine glands, an additional case of hypophyseal cachexia (Simmond's disease) is briefly reported.

O. SAPHIR.

**AORTITIS THROMBOTICA.** L. DESCLIN, Frankfurt. *Ztschr. f. Path.* **40**:520, 1930.

Seven cases of aortitis thrombotica are reported. The inflammatory process may reach the aorta from contiguous organs or tissues, or the aortitis may be the result of an acute vegetative endocarditis of the aortic valve. In the latter case, the infectious material may reach the aortic intima either by direct extension from the valvular endocardium or by an implantation on the aortic intima. It is also possible that broken, loose vegetations are carried by the blood stream directly to remote portions of the aortic intima or reach the aorta indirectly by means of the vasa vasorum and thus produce an aortitis. It is also conceivable that another primary focus of infection, aside from the endocarditis, might constitute the source of infectious emboli, which occasionally might lodge in the vasa vasorum. The author also believes that an aortitis sometimes is the primary lesion, as is an endocarditis. In the individual case, it is often impossible to establish the exact pathogenesis of the aortitis.

O. SAPHIR.

**MALFORMATION OF THE BRAIN WITH HYDROCEPHALUS AND PORENCEPHALUS.** I. WERTKIN, Frankfurt. *Ztschr. f. Path.* **40**:571, 1930.

A case of malformation of the brain is reported in a boy, aged 15 weeks. The largest portion of the cerebrum was replaced by a clear liquid. Only the lower

and posterior portions of the left hemisphere were present. The right hemisphere was almost completely converted into a saclike cavity filled with a clear liquid. The corpus callosum was not present. The anterior portions of the left hemisphere showed outspoken microgyria. In the region of the boundaries between the right temporal, occipital and parietal lobes there was a depressed area measuring from 2 to 3 mm. in diameter, from which the sulci and gyri radiated in various directions. The corpora quadragemina and the sylvian aqueduct were partly absent. The fourth ventricle was not dilated. The superior vermis of the cerebellum was not present. The branches of the vessels ran superficially and did not extend into the fissures. There were two central canals found in the region of the medulla oblongata. The hydrocephalus is explained on the basis of an atresia of the sylvian aqueduct. The various possibilities leading to these malformations are discussed.

O. SAPHIR.

HEMORRHAGIC DIATHESIS. P. MOROWITZ, Verhandl. d. deutsch. path. Gesellsch. 25:32, 1930.

The author's conclusions as to the hemorrhagic diathesis are drawn from clinical, anatomic and experimental investigations. Essentially these conclusions are that hemorrhagic diathesis results from changes in the walls of blood vessels, either by infections or by toxic, hormonal or nervous mechanisms, and that platelets play a minor rôle.

*Hemophilia.*—The prolonged coagulation time in hemophilia is not sufficient to explain the hemorrhagic tendency, for animals given injections of hirudin, by which the coagulation time is prolonged, bleed only from wounds, and not spontaneously. The author believes that the disease is associated with a vasomotor disturbance. There are no definite pathologic anatomic changes in hemophilia.

*Thrombocytopenia.*—The belief that in thrombocytopenia the giant cells of the bone marrow are sufficient in number, but defective, does not harmonize with the fact that animals poor in platelets and fibrinogen do not show hemorrhagic manifestations. The rôle of the spleen has not yet been fully determined, since in many instances the removal of the spleen does not end the bleeding. The importance of the vascular changes has stimulated the invention of an instrument by which vascular changes and the formation of thrombi can be studied in the circulating blood. This is called a thrombometer (Jürgens).

*Scurvy.*—The infectious nature of scurvy following a deficiency of vitamins is considered as the probable underlying factor and not a decrease in the cement substance, as described by Aschoff and Koch, because no endothelium is found loose in the blood, while anatomic changes are found in the blood vessels.

*Schönlein-Henoch Purpura.*—In Schönlein-Henoch purpura, an urticarial eruption that comes out in crops is followed by hemorrhagic extravasations in these areas. This is believed to be due to a toxic action on the endothelium or to anaphylaxis (Ganzmann). The symmetry in which these lesions sometimes occur suggests a central origin by the way of the vasomotor system. From then on one has capillary dilatation, stasis, exudation and bleeding.

SOL ROY ROSENTHAL.

ESSENTIAL THROMBOPENIA. SCHMINCKE, Verhandl. d. deutsch. path. Gesellsch. 25:50, 1930.

A case of essential thrombopenia is reported, in which the number of megakaryocytes in the bone marrow was markedly diminished, in spite of active granulopoiesis and erythropoiesis. In the spleen were giant cells derived from the reticulum cells. The giant cells are regarded as compensatory for the deficiency in the bone marrow. The underlying cause is believed to be a disturbance of the giant cells in the bone marrow (with decrease in the formation of platelets) rather than a destruction of the platelets in the spleen.

SOL ROY ROSENTHAL.

ANATOMIC CHANGES IN AGRANULOCYTOSIS. W. KOCH, *Verhandl. d. deutsch. path. Gesellsch.* **25:53**, 1930.

Agranulocytosis, first described as a clinical entity by Werner Schultz, occurs usually in women between 30 and 60 years of age. The attack is accompanied by high fever and icterus and at times by herpes. Angina sets in, which may go on to gangrene and paratonsillitis. The lymph follicles of the pharynx, the base of the tongue, the epiglottis, the larynx, the gums and the uvula enlarge and may suffer necrosis. The lymph nodes of the cervical and maxillary groups may swell. Necrotic conjunctivitis, gangrenous colpitis, vulvitis and proctitis may develop, as well as vesicles, papules and erysipeloid lesions of the skin. Diarrhea results from the intestinal ulcerations. The liver and the spleen are usually enlarged. Hemorrhagic diathesis is not common. The blood shows a marked decrease in the number of the white cells, the highest count being rarely over 1,800 per cubic millimeter. Mainly lymphocytes and monocytes are present. The blood platelets are normal. The red blood corpuscles are not reduced in number. Bacteriologic examination has yielded no specific organism. Death usually follows hemorrhagic bronchopneumonia or pulmonary gangrene. In 7,000 autopsies the author found 22 cases of agranulocytosis. Ulcerated and necrotic lesions were found involving all the mucous membranes, and in many instances thrush was present. These changes were probably all secondary. Necrotic foci, usually perivascular, were also noted in the liver, spleen and bone marrow. The striking feature is the paucity of reactive cellular elements. A few lymphocytes and monocytes are found, but no leukocytes. The etiologic factor is considered to be a cryptogenic infection, which is unrecognized because of lack of fever, or a toxin perhaps derived from the intestine. These bacteria or toxins destroy the leukocytes, which predisposes the body to secondary infection. With this complication, fever and the acute toxic state set in. The ulcerated and necrotic character of the lesions is due to the lack of response of the body, especially of the granulocytes.

SOL ROY ROSENTHAL.

ACUTE ALEUKEMIC "RETICULOSIS." K. TERPLAN, *Verhandl. d. deutsch. path. Gesellsch.* **25:69**, 1930.

"Reticulosis or reticulo-endotheliosis" is defined as a disease of the blood in which the reticulum cells of the liver, spleen, lymph glands and bone marrow proliferate to a marked degree. The reticulo-endothelium of the other organs may also proliferate. These changes are usually associated with a relative or absolute increase in the number of the monocytes or monoblasts (reticulo-endothelial cells) of the blood. Rarely an aleukemic form exists in which there are no characteristic changes in the blood. The author holds this to be an infectious disease, as the patients are septic, have high temperatures and die within a short period of time (eight weeks). The course is similar to that of acute leukemia. The author describes a case of an aleukemic reticulosis in a 1 year old child; a high temperature developed suddenly with generalized lymphadenopathy, anemia, hemorrhages into the skin and necrotic angina. Death occurred ten days after the onset. The blood was normal, except for the anemia. At autopsy there were a general enlargement of the lymph nodes and of the liver and pneumococcal foci in the lung. Microscopically, there was marked proliferation of the reticulo-endothelium in the lymph glands, the lung and the heart. The liver and the myocardium showed interstitial inflammatory changes. The histologic picture was very similar to that of typhoid fever because of the presence of large swollen histiocytic cells.

SOL ROY ROSENTHAL.

ON THE ORIGIN OF SO-CALLED POLYBLASTS. G. SEEMANN, *Verhandl. d. deutsch. path. Gesellsch.* **25:77**, 1930.

By vital staining of the loose connective tissue in rats with neutral red and janus green, the author demonstrates that the local histiocytes and fibrocytes are

two distinct types of cells. The former is plump and contains many coarse, neutral red-staining granules, while the latter is spindle-shaped with a few finely granular red elements. On injection of a peptone broth, a reaction is set up in the connective tissue, and in from three to five hours there is an infiltration by granulocytes and round cells. The latter are not lymphocytes, but monocytes or monocytoïd cells, derived from the blood stream. After from six to eight hours the local histiocytes proliferate, while the local fibrocytes and endothelial cells are inactive. The author concludes that the polyblasts of Maximow have two origins: that from the local histiocytes and that from the monocytes of the blood. He does not believe that lymphocytic cells produce polyblasts, but that what Maximow called lymphoblasts were really monocytoïd cells, and that the latter, not the former, are the stem cell.

SOL ROY ROSENTHAL.

### Microbiology and Parasitology

ACTINOMYCOSIS STARTING AS APPENDICITIS WITH EXTENSIVE VISCERAL INVOLVEMENT. GEORGE M. ROBSON, *Am. J. M. Sc.* **181**:692, 1931.

Two cases of actinomycosis of the liver and lungs are reported. Both followed operations for appendicitis; in each there developed a discharging abdominal sinus, symptoms in the upper abdominal region and severe pulmonary suppuration. In one case there was a generalized dissemination by the blood stream, with additional lesions in the brain, spleen and kidneys. The diagnoses were not made during life, principally because the ray fungi were not found in the discharges.

AUTHOR'S SUMMARY.

NATIVE INFESTATION WITH *DIPHYLLOBOTHRIUM LATUM* (FISH TAPEWORM).

I. PILOT and I. M. LEVIN, *Am. J. M. Sc.* **181**:710, 1931.

Five cases of native infestation by *Diphyllobothrium latum* are reported in children. All of our patients were Jews; twenty-one of twenty-six cases of native infestations collected from recent literature occurred in children from 3 to 15 years of age; sixteen of the twenty-one were Jews. Infestation in three of our cases occurred from sampling the raw fish while it was being prepared into "gefüllte fish." The other two probably developed from improperly cooked fish.

AUTHORS' SUMMARY.

LEPTOSPIRA ICTEROHEMORRHAGIAE IN WILD RATS ABOUT SAN FRANCISCO BAY. J. R. RIDLON, *Pub. Health Rep.* **46**:1, 1931.

In the kidneys of wild rats from cities on San Francisco Bay, spirochetes were found that apparently conform to the descriptions of *Leptospira icterohaemorrhagiae*. Guinea-pigs were inoculated with material from the kidneys of rats harboring the organisms and died, showing fever and jaundice of the eyes and skin before death. At autopsy they showed subcutaneous jaundice and hemorrhages of subcutaneous tissues and internal organs, which are the gross pathologic changes described by several writers as typical of infection with *Leptospira icterohaemorrhagiae*. The organisms were found in the internal organs and urine of infected guinea-pigs. Positive cultures were obtained. Guinea-pigs were infected by injection of the positive cultures. The disease was carried over in successive inoculations of guinea-pigs, both by injections of original rat material and by injections of cultures.

AUTHOR'S SUMMARY.

THE OCCURRENCE OF INTRANUCLEAR INCLUSIONS IN THE NERVE CELLS IN POLIOMYELITIS. E. W. HURST, *J. Path. & Bact.* **34**:331, 1931.

The bodies described are probably nuclear inclusions of the same order as those in herpetic encephalitis, Borná's disease, etc.; they are, however, much less

numerous, smaller and more difficult to stain electively than these other inclusions. They can be distinguished from the acidophilic masses normally present by their greater sharpness of outline and their more solid appearance and by the fact that under conditions leading to their appearance the acidophilic coagulum is wanting in whole or in large part. Their paucity and the limited time during which they can be found do not favor a study of their evolution, but it seems possible that they may represent a nuclear reaction to the attack of the virus. It is known that after intracerebral inoculation the virus does not reach the cord, at least in detectable amount, until immediately before the onset of paralysis (Fairbrother and Hurst, 1930); the bodies have never been found during the incubation period. They occur always in damaged cells but, on the other hand, they have not been seen in completely necrotic cells. In the poliomyelitis of monkeys, a considerable proportion of the anterior horn cells undergo complete necrosis within a few hours of the onset and do not necessarily pass through a phase of degeneration in which the bodies are present; indeed, the latter may develop only in cells possessing rather more power of resistance to the virus and which consequently degenerate more slowly. However this may be, they have not been seen in markedly degenerate cells in the cords of rabid monkeys or in a series of other pathologic and normal tissues. Similar bodies were present in the only human case examined, and as far as this limited evidence goes, they appear to be characteristic of the disease.

AUTHOR'S SUMMARY.

SILENT TYPHUS INFECTION. W. BARYKINE, S. MINERVINE and A. KOMPANÉEZ, Arch. Inst. Pasteur de Tunis **19**:422, 1930.

Epidemiologically, silent typhus infection is as important as the type in which symptoms appear. The silent infection may be seen in those who have and in those who have not been previously infected. This type of the disease may be suspected from an increase in the Weil-Felix reaction, but may be proved only by inoculating guinea-pigs with the blood of the patient.

INOCULATION WITH BACTERIUM GRANULOSIS. CHARLES WEISS, Arch. Inst. Pasteur de Tunis **19**:433, 1930.

Two strains apparently of *Bacterium granulosis* were isolated by Noguchi's methods and inoculated into the eyes of monkeys. In two weeks flaky granulations were noted, and conjunctivitis with marked redness and edema of the eyelids. This condition was maintained for several weeks, after which recovery occurred slowly. The original Noguchi strain was inoculated into three human volunteers and one monkey, but no reaction was observed.

M. S. MARSHALL.

SPONTANEOUS TUBERCULOSIS IN DOMESTIC ANIMALS WITH REFERENCE TO RANKE'S TEACHING. K. NIEBERLE, Beitr. z. Klin. d. Tuberk. **75**:179, 1930.

The essential thesis of this paper is that Ranke's teachings are fully confirmed by studies on the spontaneous tuberculosis of domesticated animals.

MAX PINNER.

STUDIES ON TUBERCULOUS LIQUEFACTION. W. PAGEL, Beitr. z. Klin. d. Tuberk. **76**:414, 1931.

Tuberculous liquefaction can start without the aid of cells and inflammatory processes; it may be caused by hydration of the focus or by the so-called liquor diapedesis (Ricker) from blood vessels. In the incipient stage of liquefaction, macrophages and histiocytes, and not polymorphonuclears, are prominent. Tubercle bacilli, in some instances, become visible only after the incipient stage is passed. Liquefaction and caseation are separate processes; the former supersedes the

latter as a new development. Secondary organisms do not play a causative rôle in liquefaction. In experiments on animals it was shown that specific allergy is an essential factor in the causation of liquefaction. By irradiating a focus of first infection it is possible to simulate to a lesser degree the allergic tissue reaction. Three types of liquefaction may be distinguished: (1) liquefaction in attenuated and prolonged infection, (2) allergic liquefaction and (3) nonspecifically stimulated liquefaction.

MAX PINNER.

THE LATE TUBERCULOUS PRIMARY COMPLEX IN ADULTS. E. RAGNATTI, *Beitr. z. Klin. d. Tuberk.* **76**:459, 1931.

A tuberculous primary infection may be acquired at any age; its occurrence after puberty, however, is rare. In 4,000 consecutive autopsies performed on adults, there were 36 in which a recent primary complex was found. In 72 per cent it was an aerogenous focus; in the remaining 28 per cent it was an intestinal focus. In all aerogenous foci the regional lymph nodes were characteristically involved. In every detail, primary complexes arising in adults have the same characteristics as those arising in children.

MAX PINNER.

TUBERCLE BACILLI IN THE BLOOD IN LARYNGEAL TUBERCULOSIS. E. WESSELY and E. LÖWENSTEIN, *Beitr. z. Klin. d. Tuberk.* **76**:647, 1931.

The recent method of Löwenstein for the isolation of tubercle bacilli from the blood was used. Of forty-seven patients with laryngeal tuberculosis, twenty-six yielded tubercle bacilli from the blood (only one examination for each patient).

MAX PINNER.

CHEMICAL DIFFERENTIATION OF HEMOLYTIC AND NONHEMOLYTIC STREPTOCOCCI. L. AVILÉS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **69**:433, 1931.

By means of the berlin blue reaction, it could be shown that hemolysis by certain streptococci may be due to destruction of hemoglobin in the bodies of the streptococci, because the colony itself became blue, while the hemolytic zone was unchanged. In the case of nonhemolytic streptococci, on the other hand, the colony itself remained white, while a blue zone due to the liberation of inorganic iron developed around the colony. This difference was marked on chocolate agar plates, more so on plates made with human blood than on plates made with beef or sheep blood.

GAS-FORMING VARIANTS OF THE TYPHOID BACILLUS. C. SONNENSCHNEIN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **69**:454, 1931.

The claims that the typhoid bacillus may develop gas-forming variants could not be substantiated.

PERORAL INFECTION OF GUINEA-PIGS IN TUBERCLE BACILLI. J. ORSKOV and K. A. JENSEN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:146, 1931.

Following oral introduction of virulent tubercle bacilli, infection develops step by step by way of the lymphatics and lymph nodes. The first localization is in the regional lymph nodes.

CULTIVATION OF TUBERCLE BACILLI FROM THE BLOOD ACCORDING TO LÖWENSTEIN. A. FISCHER, *Ztschr. f. Tuberk.* **58**:331, 1930.

Seventy-seven specimens of blood from tuberculous patients in various stages of the disease were cultured, according to the recently published technic of Löwenstein, and from thirty-one of them tubercle bacilli were obtained in pure culture.

MAX PINNER.



THE FILTRABLE ELEMENTS OF THE TUBERCULOSIS VIRUS. A. CALMETTE and J. VALTIS, *Ztschr. f. Tuberk.* **58**:402, 1930.

Filtrates from young cultures of the tubercle bacillus, from tuberculous organs or from excreta cause, after subcutaneous injection, lymphoglandular swellings in guinea-pigs. The involved nodes that do not show tuberculous lesions contain few acid-fast rods and granules (from three days to eight weeks after the injection). Such nodes produce, after a few direct animal transfers, typical tuberculous lesions. These filtrable elements are capable of passing through the placenta both in experimental animals and in human beings. The filtrable virus can be cultivated in vitro, and under this condition its development into typical tubercle bacilli can be observed. Transplants of the virus have been unsuccessful as yet. The filtrable virus can be demonstrated most readily by injecting it directly into lymph nodes or into the spinal canal. The authors conclude that the disease tuberculosis, characterized by specific changes in the tissues and by the presence of tubercle bacilli, is only the chronic end-stage of an infection caused by the filtrable virus, which per se does not produce tubercles, but which may exert marked toxic influences. This virus is considered the etiologic agent in such diseases as purpura rheumatica, erythema nodosum, sarcoids, papulo-necrotic tuberculids, typhobacillosis Landouzy and the "granulémie" (i. e., miliary tuberculosis). It is suggested that all diseases caused by the filtrable virus be called "prebacillary granulemia," and those caused by true tubercle bacilli, "bacillosis."

MAX PINNER.

SECONDARY COLONIES, AUTOLYSIS AND LYSIS IN CULTURES OF TUBERCLE BACILLUS. E. A. SCHNIEDER, *Ztschr. f. Tuberk.* **59**:18, 1930.

In culturing tubercle bacilli the formation of secondary colonies and lytic phenomena can be observed in a similar way as with other micro-organisms. Secondary colonies and lysis appear to be closely related occurrences; they are both enhanced by unfavorable mediums.

MAX PINNER.

A TUBERCULOUS PRIMARY FOCUS WITH ATYPICAL HISTOLOGIC STRUCTURE. M. PINNER, *Ztschr. f. Tuberk.* **59**:130, 1930.

A primary tuberculous focus in the lung is described that showed, in addition to the usual type of encapsulation, the formation of a diffuse intra-alveolar connective tissue.

MAX PINNER.

EXPERIMENTAL TUBERCULOSIS. W. A. LUBARSKI and A. F. KORSCHINSKAJA, *Ztschr. f. Tuberk.* **59**:252, 1931.

Following intravenous injection of tubercle bacilli into the rabbit, the lungs show the most massive involvement. After subcutaneous injection of tubercle bacilli into the guinea-pig, the lungs and spleen are mostly involved. The number of tubercle bacilli is largest in the lungs, and considerably less in the spleen and liver. Tubercle bacilli must be destroyed in the organs. This destruction is most marked in the liver. The organs of white mice have the greatest capacity to destroy tubercle bacilli. In pure cultures from organs of infected animals, three different types of colonies are observed.

MAX PINNER.

HOHN'S MEDIUM AND THICK SEEDING OF SEDIMENT. L. FRANKL, *Ztschr. f. Tuberk.* **59**:262, 1931.

When the sediment is seeded thickly on Hohn's medium, tubercle bacilli are demonstrable in about 50 per cent of the positive cases within the first few days, while after thin seeding the first colonies become visible only after from eight to thirty days.

MAX PINNER.

THE SIGNIFICANCE OF THE SILICIC ACID CONTENT OF THE BLOOD IN TUBERCULOUS PATIENTS. R. H. KRANZFELDER, *Ztschr. f. Tuberk.* **59**:398, 1931.

The amount of silicic acid in normal persons (twenty-three) is 16 mg. per hundred cubic centimeter of blood; within four months the variations observed were less than 4 per cent. In tuberculous patients the values are, on the average, 18 per cent higher. The silicic acid content of the blood increases readily after administration by inhalation; it may increase more than the amount administered explains. Patients with high silicic acid content (without previous administrations) have, in general, a poorer prognosis than patients with lower values.

MAX PINNER.

THE RELATION OF CELL GRANULES TO PHAGOCYTOSIS OF BACTERIA. H. HAZATO, *Jap. J. Exper. Med.* **9**:67, 1931.

Bacteria taken up and digested in histiocytes are regularly included in neutral red granules or vacuoles, in which they undergo digestion. When neutral red granules or vacuoles have previously been impregnated with sepia or carmine the majority of phagocytosed bacteria are capable of finding their way into the preformed sepia or carmine granules or vacuoles to be digested therein, but never into degenerative ones. When a histiocyte phagocytoses and digests bacteria, the neutral red granules or vacuoles, as well as the fat-droplets or lipin of the cell, show a gradual increase in number and in size, and the cell itself is correspondingly enlarged. With the distinct increase in number and in size of the neutral red granules or vacuoles, there is a gradual decrease of the number of mitochondria. At this stage the mitochondria of the histiocyte show a greater degree of osmic reaction than normal.

AUTHOR'S SUMMARY.

RICKETTSIA ORIENTALIS, THE CAUSE OF TSUTSUGAMUSHI DISEASE. M. NAGAYO and others, *Jap. J. Exper. Med.* **9**:87, 1931.

When the virus of Tsutsugamushi disease is introduced into the anterior chamber of the eye in rabbits and other animals it causes a peculiar lesion, one of the characteristics of which is the accumulation of minute organisms in the endothelial cells of Descemet's membrane. These organisms correspond to those found in patients suffering from the disease and in experimentally infected animals. The infected endothelial cells carry the infection with them when introduced into other animals. In cultures of the cells in vitro, the micro-organism, which is designated *Rickettsia orientalis*, proliferates freely. The article is illustrated with forty-seven figures, many in colors.

TOXIN OF THE SHIGA BACILLUS. H. KAWAMURA, *Kitasato Arch. f. Exper. Med.* **8**:1, 1931.

The specific dysentery toxin is produced within the bacillary bodies and released into the medium after the bacilli are dead. It is not a secreted toxin. The toxin weakens after a short time. The combining power of the toxin and the antitoxin increases with the increase in toxicity after the death of the bacilli and decreases as the toxin weakens. The toxin does not produce a true toxoid. A strong toxin can be developed in a peptone-free synthetic medium. A 0.5 per cent formaldehyde solution weakens the toxin. The bacilli treated with formaldehyde retain their antigenic properties, but the autolysates treated with formaldehyde have very weak antigenic power. When treated with toluene or phenol, the old preparations show no decrease in toxicity, but have little antigenic power. Toxin treated by heat at 80 C. or above and by hydrogen dioxide loses both toxicity and antigenic power.

EDNA DELVES.

THE ETIOLOGY AND PATHOGENESIS OF ACTINOMYCOSIS. CARL NAESLUND, *Acta path. et microbiol. Scandinav.*, Supp. 6, 1931.

The main outcome of the work recorded in the German language in this monograph of 150 pages is a well supported proposal to simplify and stabilize the nomenclature of actinomycosis and its agents. It is proposed to divide the pathogenic ray fungi (*Actinomyces*, *Streptothrix*, *Nocardia*, etc.) into two groups, the anaerobic and aerobic, and to call the first group *Actinomyces A* and the corresponding diseases actinomycosis A, while the second group is called *Actinomyces B* and the corresponding diseases actinomycosis B.

## Immunology

THE ANTIGENIC PECULIARITIES OF STRAINS OF SPIROCHAETA PALLIDUM. W. M. ARISTOUSKY and A. J. WSOROW, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **69**:351, 1931.

*Spirochaeta pallidum* contains species-specific and type-specific antigens which, so to speak, compete with one another in their action on the organism.

ANAPHYLACTIC SHOCK FROM INTRACEREBRAL INJECTION OF ANTIGEN. A. SCHWARZMANN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **69**:379, 1931.

The shock after intracerebral injection of antigen into sensitized guinea-pigs is due to the passage of the antigen into the blood. The mechanism of the shock is the same as in intravenous injection of the antigen; it requires about four times as much antigen to produce shock on intracerebral injection of antigen as on intravenous.

PROTECTIVE INOCULATION AGAINST EPIDEMIC POLIOMYELITIS. R. KRAUS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **69**:424, 1931.

Monkeys can be protected against experimental poliomyelitis by injection of phenolvirus, which is obtained by adding from 1 to 1.5 per cent of phenol to emulsions of poliomyelitic virus and filtering through paper. The phenol kills the virus, or greatly reduces its virulence, without destroying its antigenic properties.

THE HETEROGENETIC ANTIGEN OF THE SHIGA BACILLUS. K. MEYER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **69**:499, 1931.

The heterogenetic antigen is more or less firmly united with the specific carbohydrate.

EFFECTS OF OTHER BACTERIA ON THE RESISTANCE TO INFECTION WITH TYPHOID BACILLI. S. NUKADA and S. ARIFUKU, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:1, 1931.

In guinea-pigs immunization with certain bacteria, e. g., pneumococci, increases the resistance to fatal infection with typhoid bacilli, owing perhaps to alterations in the cells of the tissues.

THE ANTIGENS OF THE SHIGA BACILLUS. M. SISLER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:48, 1931.

The carbohydrate from the Shiga bacillus acts as antigen only when it contains human blood antigen as well as the Forssman antigen. Such a carbohydrate reacts only with serum against the Shiga bacillus that in addition to protein antibodies also contains heterogenetic antibodies.

FROM AUTHOR'S SUMMARY.

THE RÔLE OF ALCOHOL-SOLUBLE CONSTITUENTS OF MILK IN ITS SEROLOGIC REACTIONS. M. DEHIO, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**: 58, 1931.

By immunizing rabbits with extract of milk in 50 per cent alcohol, an anti-serum was developed that reacted with the homologous antigen as well as with the native milk. Following treatment of rabbits with extract of milk, in 96 per cent alcohol antibodies did not develop, at least not regularly. Aqueous and alcoholic solutions of sodium caseinate reacted with antimilk serum. The action of antimilk or anticasein serum on heterologous milk may depend, at least in part, on the alcohol-soluble constituents in milk.

FROM AUTHOR'S SUMMARY.

ACTION OF LYSOZYME ON *MICROCOCCLUS LYSODEIKTICUS*. O. ANDERSEN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:90, 1931.

This article describes the results of studies of the action of lysozyme from egg-white on *Micrococcus lysodeikticus*, a sarcina. Pathogenic bacteria and animal cells are not subject to the action of lysozym.

THE ACTION OF BCG. K. A. JENSEN and J. ORSKOV, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:155, 1931.

Following the oral introduction of BCG into guinea-pigs there was no general invasion of the lymphatics by tubercle bacilli, but localization in the most peripheral parts of the regional lymphatic system. Does this process suffice to produce a fairly constant immunity? The results in animals indicate that the answer to this question is no.

THE PRODUCTION AND PURIFICATION OF TUBERCULIN. E. MASCHMANN and E. KÜSTER, *Ztschr. f. Tuberk.* **59**:225, 1931.

Sauton's protein-free medium is very suitable for the production of a potent tuberculin. Tuberculin prepared with Sauton's medium shows reactions that are characteristic for proteins and carbohydrates. The specifically active substance diffuses easily through animal membranes. By acidifying the tuberculin before or after dialysis, high molecular proteins are removed without diminishing noticeably the potency of the tuberculin. The specific substance is easily adsorbed by kaolin, particularly in acid reaction. The adsorbed tuberculin can be liberated from the kaolin by very dilute ammonia; the yield is good and constant. By this process the strength of the tuberculin is increased twenty-one times, compared with the original tuberculin. The finished products have a positive biuret and ninhydrin reaction; some show a positive Millon reaction; none show a tryptophan or Molisch reaction. For the specific action the following constituents are unnecessary: the carbohydrates, those proteins that are precipitated in acid solution and those proteins that produce a positive Millon and tryptophan reaction. It must be determined whether the specific factor is a polypeptide or whether it belongs in another chemical class. A pure carbohydrate was isolated that is identical with glycogen.

MAX PINNER.

THE PROTECTIVE ACTION OF THE BCG VACCINE. A. I. TOGUNOWA, W. N. ARCHANGELSKY and S. L. BAIDAKOWA, *Ztschr. f. Tuberk.* **59**:425, 1931.

Guinea-pigs and rabbits given subcutaneous injections of BCG vaccine at once develop a relative immunity against a later subcutaneous or intra-ocular infection with virulent bacilli. The duration of this immunity is dependent on the animal species and on technical detail.

MAX PINNER.

## Tumors

THE ORIGIN AND NATURE OF MENINGEAL TUMORS. P. BAILEY and P. C. BUCY, *Am. J. Cancer* **15:15**, 1931.

The authors are of the opinion that meningeal tumors, whatever their origin, are of the nature of connective tissue and are not gliomatous. This is in disaccord with the opinion of those who claim that the leptomeninx is of neuro-ectodermal origin. They found at least nine distinct histologic types of tumors arising in the meninges. The relationship of these tumors to each other is represented by them in a diagram. They further believe that the fibroblast is usually not the typical cell of these tumors as it is claimed by Mallory and Penfield.

B. M. FRIED.

MALIGNANT MIXED TUMORS OF THE THYROID GLAND WITH SKELETAL MUSCLE FIBERS. EDWIN F. HIRSCH, *Am. J. Cancer* **15:55**, 1931.

A malignant tumor of the thyroid gland and its metastasis in many of the visceral tissues of the body contained embryonal skeletal muscle fibers, undifferentiated cells and large fused-cell aggregates. Some of the fused-cell aggregates contained hyaline masses with the staining qualities of thyroid colloid. These cell masses, however, were not identified conclusively as abortive thyroid acini, although some resembled such structures closely.

AUTHOR'S SUMMARY.

A STATISTICAL STUDY OF THE OCCURRENCE OF SPONTANEOUS TUMORS IN A LARGE COLONY OF RATS. M. R. CURTIS, F. D. BULLOCK and W. F. DUNNING, *Am. J. Cancer* **15:67**, 1931.

The article represents a detailed statistical analysis of a large rat colony in respect to the occurrence of spontaneous neoplasms.

B. M. FRIED.

EMBRYONAL NEPHIROMA IN THE CHICKEN. F. D. MCKENNEY, *Am. J. Cancer* **15:122**, 1931.

The two rare tumors reported belong to the group of neoplasms which, when found in human beings, are known as Wilms' tumors. The tumors were growing within the kidney without having any capsule and not being attached to the spinal column. No metastasis could be demonstrated, although the histologic picture of the new growths was that of malignant tumors.

B. M. FRIED.

BIOCHEMICAL STUDIES OF MALIGNANT CONDITIONS. JOSEPH H. ROE and HELEN M. DYER, *Am. J. Cancer* **15:125**, 1931.

Dextrose tolerance test showed a lowered carbohydrate tolerance in a fowl bearing the Rous sarcoma no. 1. The reduced carbohydrate tolerance is probably secondary to changes produced in the organs regulating carbohydrate metabolism, either by metastases or by toxemia due to the malignant growth.

The glycogenolytic activity of the blood of hens in which the Rous sarcoma no. 1 had developed showed no variation from that of normal controls.

The blood of a fowl bearing the Rous sarcoma no. 1 has a glycolytic activity about twice as great as that of a normal fowl. This is a primary change resulting apparently from an overflow into the blood stream of the glycolytic enzyme, which Warburg showed to be in tumor tissue in greatly increased amounts.

An examination of the glycolytic activity of the blood of sixteen patients with malignant tumors gave values within normal limits. The determination of the glycolytic activity of the blood is, therefore, of no clinical value in studying

malignant conditions. The discrepancy between the glycolytic activity of the blood of hens with Rous sarcoma no. 1 and that of the blood of patients with malignant conditions is probably due to the greater ratio of tumor tissue to normal tissue in the fowl with the sarcoma.

AUTHORS' SUMMARY.

THE INFLUENCE OF EXTRACTS OF SUPRARENAL CORTEX ON THE GROWTH OF CARCINOMA, SARCOMA AND MELANOMA IN ANIMALS. K. SUGIURA, *Am. J. Cancer* **15**:129, 1931.

The toxic action of extracts of suprarenal cortex of the sheep and cattle on the Flexner-Jobling rat carcinoma, the Sugiura rat sarcoma, the Bashford mouse carcinoma no. 63, a transplantable mouse melanoma and the Rous chicken sarcoma has been investigated. The author found that single or repeated subcutaneous or intramuscular injections of an alcoholic, ether, aqueous or glycerin extract of the cortical tissues has no apparent influence on the growth of transplanted tumors. Injection of choline solution or feeding with sodium nitrite chemicals known to cause a prompt fall of blood pressure failed to show any inhibiting effect on tumor growth. Extracts from the adenomatous growths of sheep suprarenal glands which are almost completely free from medullary tissue, produced no effect on the growth of neoplasms. The repeated subcutaneous injection of extracts of the suprarenal glands of sheep, of immune and normal young rats and of suckling rats does not affect the growth of rat and mouse carcinoma. Finally, fresh sheep suprarenal cortex or its extract failed to destroy cancer cells *in vitro*.

B. M. FRIED.

OCCUPATIONAL NEOPLASTIC DISEASES. C. D. HAAGENSEN, *Am. J. Cancer* **15**:641, 1931.

Haagensen, in accord with other workers, found a high incidence of skin cancer among workers in gas works and petroleum refineries. He also found that occupational exposure to lubricating oil, as in the case of mechanics and machinists, predisposes to cancer of the external genitalia—a predilection as to anatomic site which seems best explained by the uncleanness of these organs common in laborers. Likewise, an excess of cutaneous cancer among outdoor workers affecting the face and the lip shows the importance of sunlight in the etiology of cutaneous cancer.

Metastases occurred in 63 per cent of cases of occupational roentgen cancer, which suggests that this type of cancer is more malignant than some workers have reported it to be. The author could find no association between cancer of the lung and bladder with occupation. In general, occupational exposure is most likely only one of the multiple etiologic factors of cancer.

B. M. FRIED.

THE SUPRARENAL AND TUMOR GROWTH. W. H. WOGLOM, *Am. J. Cancer* **15**:704, 1931.

The suprarenal glands of rabbits that have been inoculated with carcinoma "63" or sarcoma "180" have no demonstrable inhibitory effect on the growth of these tumors in mice.

B. M. FRIED.

THE INFLUENCE OF AN AQUEOUS EXTRACT OF SUPRARENAL CORTEX ON THE GROWTH OF CARCINOMA, SARCOMA AND MELANOMA IN ANIMALS. K. SUGIURA, *Am. J. Cancer* **15**:707, 1931.

Repeated subcutaneous or intramuscular injections of aqueous extracts of sheep suprarenal cortex, which were prepared according to the method described by Coffey and Humber, failed to retard or inhibit the proliferation of carcinoma, sarcoma or melanoma cells. These tumors, whether young or old, grew normally

and killed the hosts in the usual manner in spite of the administration of large or small doses of cortical extracts. There was no evidence that injection of the aqueous extracts of cortical tissues accelerated the growth of these transplantable tumors, nor did the injection of the aqueous extracts of sheep suprarenal cortex have any appreciable effect in checking the development of metastases in visceral organs of sarcoma-bearing chickens. Metastatic growths were observed as frequently in the treated as in the untreated animals.

B. M. FRIED.

IMMUNITY TO TRANSPLANTABLE RAT TUMORS. RAYMOND E. GARDNER and ROSCOE R. HYDE, *Am. J. Hyg.* **13**:649, 1931.

Groups of rats from a stock susceptible to transplants of a spindle cell sarcoma (Walker's tumor no. 1) and of a carcinoma (Walker's tumor no. 256) were given preliminary treatment with a variety of substances before tumor inoculation. Fresh whole chicken blood given subcutaneously exerted a definite influence in preventing the growth of both sarcoma and carcinoma transplants. Nonhemolytic heat-dried whole chicken blood was successful in a smaller proportion of cases of both sarcoma and carcinoma. Whole dried chicken blood produced no resistance to the growth of either of the tumors. Feeding of whole chicken blood also produced no effect. A larger proportion of animals inoculated subcutaneously with blood-free chicken embryo skin failed to develop sarcoma than did the controls, but this was not so in the case of carcinoma. A similar difference was noted when vaccine virus was used. Vaccine virus was not recovered after inoculation into either sarcoma or carcinoma from nine to fourteen days later, although the virus is recoverable from normal tissue. The treatment of rats with dried carcinomatous tissue produced no resistance to subsequent inoculations of the same tumor.

Rats of susceptible stock proved refractory to sarcoma transplants were resistant to carcinoma transplants in 46 per cent of cases. No increase in the susceptibility of rats naturally resistant to subcutaneous implantations of sarcoma could be noted after making implantations in the brain.

In general, when any of the treatments produced fewer instances of tumor development than occurred in the corresponding control group, there was a retardation of tumor development in those treated animals in which tumor growth did take place.

P. H. GUINAND.

DOES CARCINOMA OF THE DUODENUM EVER ARISE FROM DUODENAL ULCERS? J. WILLIAM HINTON, *Am. J. M. Sc.* **181**:843, 1931.

During the years 1928, 1929 and 1930, 324 ulcers were observed at the gastroenterologic clinic of the fourth medical and surgical divisions of Bellevue Hospital. Of this number, there were 269 duodenal lesions, 34 gastric, 14 pyloric and 6 double ulcers, meaning a lesion in the stomach and duodenum. With the marked frequency of duodenal ulcers, it is difficult to explain why these cases do not undergo carcinomatous degeneration. Primary carcinoma of the duodenum is occasionally seen. Clinically, one can disregard the possibility of a duodenal ulcer ever taking on malignant degeneration.

AUTHOR'S SUMMARY.

PRIMARY CARCINOMA OF THE DUODENUM. JACOB MEYER and DAVID H. ROSENBERG, *Arch. Int. Med.* **47**:917, 1931.

Four cases of primary carcinoma of the duodenum are reported. Emphasis is placed on the difficulty of clinical recognition and of the necessity of carefully studying, clinically as well as histologically, all cases of duodenal stenosis. A critical analysis may reveal a greater incidence of primary duodenal carcinomas.

AUTHORS' SUMMARY.

PINEALOMAS. JOSEPH H. GLOBUS and SAMUEL SILBERT, Arch. Neurol. & Psychiat. 25:937, 1931.

As the structure of tumors in the region of the pineal body (pinealomas) varies greatly, Globus and Silbert attempted to trace the formation of these tumors to embryonal rests and thus "find a basic morphologic pattern that would link all tumors in the pineal region, no matter how divergent their gross or histologic appearance." They studied pineal bodies of nine fetuses of various ages (from 5½ months to 8½ months), of eighteen infants from 1 to 24 days old, of twenty-five infants and young children between the ages of 1 month and 2½ years, of twenty-two older persons ranging up to the age of 72 years, and seven tumors; a total number of seventy-seven pineal bodies was studied. Among the several methods used were those of Bielschowsky, Cajal and Hortege's modification by Globus. In all tumors (except one), some phase of the basic pattern common to the developing pineal body during the first four months of postnatal life was found. The pattern consisted of streams of small deeply staining cells that broke up the pineal territory into irregular circles or squares. In the latter, large cells were found that stained less densely and assumed the character of parenchymal cells. The small cells were in some areas assuming the character of connective tissue. The glandular structure of the pineal body seemed unquestionable, especially about the sixth month of the intra-uterine life. In some cases the pattern was less typical. Glial or nerve elements did not play any rôle in the structure of the pineal body. The histologic nucleus of various types of tumors of the pineal region is furnished by the microscopic appearance of the pineal body in the third or fourth month of postnatal life.

GEORGE B. HASSIN.

CALCIFICATION IN GLIOMAS, CLEMENT B. MASSON, Bull. Neurol. Inst., New York 1:314, 1931.

One hundred and thirty-one gliomas showed on roentgen examination calcification in 12.97 per cent of patients. From the character and the distribution of the shadows seen in x-ray films, it is seldom possible to differentiate between the various types of glioma, an astrocytoma from a spongioblastoma, for instance, nor is it possible to differentiate the gliomas from the meningiomas. Calcification is not rare in rapidly growing, malignant gliomas (spongioblastomas) and the area of calcification may be limited to a part of the tumor, of which the center is already involved.

GEORGE B. HASSIN.

DERMOID OVERLYING THE CEREBELLAR VERMIS WITH A REVIEW OF THE LITERATURE ON INTRACRANIAL DERMoids. SAMUEL BROCK and DOROTHY A. KLENKE, Bull. Neurol. Inst., New York 1:328, 1931.

This type of tumor has no characteristic signs or symptoms. The diagnosis is made by roentgenogram, which in the authors' case showed the tumor in the posterior fossa, overlying the vermis and separating the two cerebellar hemispheres. The tumor was cystic and contained many long, dark hairs and numerous calcareous plaques which varied in size from that of a millet seed to that of a peanut. Intracranial dermoids are rare.

GEORGE B. HASSIN.

ROENTGEN CARCINOMA AND SARCOMA. F. W. MULSOW, J. A. M. A. 96:2030, 1931.

The development of sarcoma on one finger and a year later of carcinoma on another finger is reported in the case of a physician, aged 57 years, who had been repeatedly exposed to roentgen rays for about twelve years.



THE EFFECT OF CERTAIN INTERNAL SECRETIONS ON THE GROWTH OF CANCER.  
F. BISCHOFF, L. C. MAXWELL and H. J. ULLMAN, *J. Biol. Chem.* **92**:80, 1931.

The rate of growth of a sarcoma transplanted into rats after adrenalectomy and castration was not significantly different from the growth observed in normal rats. Transplants of the sarcoma into splenectomized rats appeared to increase in size three times as rapidly as in normal rats. Splenectomy and adrenalectomy were without effect on the growth rate of subsequently transplanted carcinomas. Applications of x-rays to the head (for destruction of the pituitary body) were followed by a retardation of the rate of tumor growth. Injections of synthalin and thyroxin were without effect.

ARTHUR LOCKE.

EXPERIMENTAL TAR TUMORS IN THE WHITE RAT. A. F. WATSON, *J. Path. & Bact.* **34**:301, 1931.

Skin tumors were obtained in two male and two female rats from an original litter of six males and five females by means of the periodical treatment of the skin with a gas works coal tar, each application of tar being preceded by treatment of the skin with a petroleum ether extract of rat tissues. Under the particular experimental conditions adopted, the tumors appeared during the period from the 415th to the 551st day after the first application of tar. The tumors of the males were squamous cell carcinomas, and once initiated grew vigorously, one animal showing extensive metastases in the lungs, both kidneys and the pleural cavity. Inoculations of the tumor tissue from these animals into normal rats were unsuccessful. The tumors of the females grew less vigorously, and in only one case could any histologic evidence of local infiltrative growth be obtained.

AUTHOR'S SUMMARY.

PRIMARY CARCINOMA OF BRONCHUS. E. E. ATKIN, *J. Path. & Bact.* **34**:343, 1931.

The observations in this paper are based on autopsies in eighty cases of primary bronchial carcinoma in men and thirteen women. The mean age of the male patients with primary carcinoma of the bronchus is higher when it is a squamous cell growth than when the cells are of other types, the respective mean ages being 51 and 43.3 years in the present series. Carcinoma does not appear to arise in one bronchus more frequently than in the other. The order of frequency of occurrence of metastases in the abdominal organs was liver, pancreas, kidney, suprarenals and spleen. In about half the cases no metastases at all were found. The squamous cell carcinomas exhibit a great tendency to necrosis and liquefaction. All the cases of definite cavitation found post mortem in the growth were in squamous cell tumors. The possibility that secondary deposits in the opposite lung are due to aspiration via the bronchus is suggested. All such metastases in the present series occurred in cases of squamous cell carcinoma with a marked tendency to liquefaction.

AUTHOR'S SUMMARY.

MALIGNANT PHAEOCHROMOCYTOMA OF THE ADRENALS. E. S. J. KING, *J. Path. & Bact.* **34**:447, 1931.

A hitherto undescribed form of pheochromocytoma of the suprarenals is reported. The growths were solid and bilateral. Cells of two types were present: typical pheochrome cells and anaplastic cells not giving the typical chrome stain, but showing all transitions from the typical cells. Multiple metastases were present in the liver, lungs, bowel, bones, skin and aortic glands. The structure of most of these was the same as the anaplastic areas of the suprarenals, but some showed both this and typical pheochrome tissue.

AUTHOR'S SUMMARY.

ANGIORETICULO-XANTHOMA OF THE RETINA (VON HIPPEL AND LINDAU DISEASE). LOUIS BERGER and ARTHUR VALLÉE, *Ann. d'anat. path.* 8:313, 1931.

The diseases described by Hippel, angiomas of the retina, and that described by Lindau, angiomas of the cerebellum and of the medulla, are the same condition with a different localization.

Berger and Vallée describe in detail an angiomatous tumor of the retina which they regard as originating from the "reticulo-endothelium" and in which a large part of the new growth had undergone a xanthomatous transformation. They, therefore, designate the tumor as angioreticulo-xanthoma. The angioma in their case varied from that of Hippel in that it invaded all the elements of the eye. The reticular nature of the growth was evidenced by the marked development of a reticulum impregnated with silver nitrate and by the abundance of intercapillary cells which were undergoing xanthomatous, reticular and "pseudo-gaucherian" transformation. The neuroglial proliferation which often accompanies the angioreticulomas does not represent an essential part of the tumor, being nothing other than a reaction to the growth. The disease occurred in a girl 12 years old. The tumor, which was diagnosed by the clinician as a glioma of the retina, was removed, and after fifteen months there was no recurrence of the disease.

The article is accompanied by eleven good drawings, five of which are in colors. The bibliography is complete.

B. M. FRIED.

TISSUE ANOMALIES AND THEIR RELATIONSHIP TO SOME TUMORS OF THE OVARIES. R. MEYER, *Arch. f. Gynäk.* 145:2, 1931.

Thirty-two photomicrographs are given, illustrating seminomas, aplasia of the ovary, granulosa-cell tumors, tubular adenomas, paragangliomomas and tumors derived from suprarenal ovarian rests ("hypernephroid tumors"). Emphasis is laid on the origin of ovarian neoplasms from embryonal dysplasias, either from incomplete formation of certain tissue elements or from their lack of proper retrogression. Tissue anomalies present in the ovaries of the cow and anthropoid apes are reported, and their significance in connection with the formation of certain tumors in man are discussed.

LAWRENCE PARSONS.

TUMORS DEVELOPING AFTER X-RAY TREATMENT OF BONE AND JOINT TUBERCULOSIS. H. KÜTTNER, *Arch. f. klin. Chir.* 164:5, 1931.

Küttner's interest in this subject was aroused by the observation of two cases in three years. One of his patients was a girl, aged 11 years, who, at the age of 5 years, had a tuberculosis of the knee for which she received some stretching operations and a total of five skin units of x-ray therapy in twelve sittings. The amputated femur was involved in a polymorphocellular sarcoma, and recurrence followed in the stump. Ten additional case reports, from the German literature, are cited to strengthen the theory that the roentgen irradiation is the most plausible factor to which to ascribe the development of such tumors, although trauma and the tumor-producing stimulus of tuberculosis are also considered. The tumors noted in the eleven cases were spindle cell sarcomas (two), polymorphonuclear cell sarcomas (six), giant cell sarcoma (one) and enchondromas (two). Six males and five females were in the series; eight had tuberculosis of the knee, two of the elbow and one of the hand. Ten patients were from 11 to 23 years old, while one (the patient with the hand involvement) was 39. An interval of from two to eleven years had elapsed between the time of healing of the tuberculous lesion and the appearance of the malignant tumor. The original diagnosis of tuberculosis seemed correct in all instances. One patient who had both a knee and hip involvement received x-ray treatment for his knee but not for his hip, and subsequently a sarcoma of the knee developed. Straying from his subject to cite evidence, Küttner brings in sarcomas and carcinomas of the face which develop on lupus following treatment with the x-rays.

A second type of tumor reported is a myeloma in a man 44 years old who had numerous x-ray treatments for a thoracic tuberculous spondylitis over a period of eighteen years.

GEORGE RUKSTINAT.

A CASE OF A TERATOID TUMOR OF THE HYPOPHYSEAL REGION. G. J. DU MARCHIE SARVAAS, Frankfurt. *Ztschr. f. Path.* 40:210, 1930.

In a boy, 12 years of age, a tumor was found in the region of the sella turcica. The tumor, which extended through the nares to the outside, had led to an open communication between the orbit, the nasal cavity and the cranial cavity. The hypophysis could not be made out. The tumor was connected, however, with the infundibulum of the hypophysis. Histologically, it consisted of connective tissue, glia fibers, which contained pigment, bone, cartilage, blood vessels, fat tissue, smooth muscle fibers, different types of epithelium, nervous tissue and embryonal tissues. Besides the tumor, autopsy revealed an atrophy of the thymus and both suprarenals. The author believes that this tumor should be classified among teratomas rather than as epignathus because of the location of the tumor, because of the absence of any parts of well developed organs, such as extremities, and because of the tumor-like growth. The relation of the tumor to the hypophysis was not studied because the hypophysis could not be located.

O. SAPHIR.

HAMARTOMAS OF THE INTRAHEPATIC BILE DUCTS AND THEIR RELATIONS TO CYSTIC LIVER. W. AUERBACH, Frankfurt. *Ztschr. f. Path.* 40:272, 1930.

The liver of a 69 year old woman contained many green and brownish-green nodules, not exceeding 8 mm. in diameter. The nodules showed a smooth cut surface and were well circumscribed. Histologically, small cysts were found embedded in a dense connective tissue which was poor in nuclei. The cysts were lined by one row of high cylindric cells and contained an amorphous greenish-brown material. A basal membrane was not clearly distinguishable. The wider the cysts, the more flattened was the lining epithelium and the more did the lining cells resemble endothelial cells. A few cystic portions were actually lined by endothelial cells. These cysts were filled with red blood cells and resembled structures found in hemangiona. No connections could be made out between the cysts and the adjacent bile ducts. The author classifies these cysts as hamartomas which for some reason did not develop fully into a cystic liver, but remained stationary.

O. SAPHIR.

TUMORS OF THE NEUROMYO-ARTERIAL GLOMUS (MASSON). M. HOPF, Frankfurt. *Ztschr. f. Path.* 40:387, 1930.

The neuromyo-arterial glomus, which was described by Masson as an organ that regulates the temperature and blood pressure, sometimes gives rise to a small benign tumor called angioneuromyoma. This tumor formerly was called angiosarcoma, endothelioma, etc. Clinically, this growth is characterized by a history of trauma, slow growth and intense pain. It is found in the region of the extremities. The tumor is soft, measuring up to 2 cm. in diameter, bluish red, and somewhat resembling an angioma. It is always well circumscribed. Microscopically, there are many vessels, the walls of which consist of epithelioid cells, smooth muscle fibers and nonmedullated nerve fibers. The tumor is surrounded by a connective tissue capsule that distinguishes this type of tumor from an angioma. The author reports four such cases.

O. SAPHIR.

PRIMARY SARCOMA OF THE PERICARDIUM. G. BODON, Frankfurt. *Ztschr. f. Path.* 40:417, 1930.

Carcinoid of the skin, squamous cell carcinoma of the bronchus, hypernephroma of the right kidney and osteochondroma of the lung are reported in a man aged 89.

O. SAPHIR.

PRIMARY SARCOMA OF THE PERICARDIUM. G. BODON, Frankfurt. *Ztschr. f. Path.* **40**:431, 1930.

Primary sarcoma of the pericardium is reported in a 65 year old man. Histologically, the tumor consisted of round cells of the size of lymphoblasts which diffusely infiltrated the pericardium. The cells showed large, round, dark-stained nuclei which were surrounded by a small mass of cytoplasm. Many nuclei showed a crescent shape and indentations which were taken as various forms of amitotic division. Between these cells, a fine reticulum was noted, which surrounded, in some fields, individual cells. There were many vessels found. The tumor cells invaded the myocardium and the subepicardial fat tissue. The case showed, in addition, a meningioma.

O. SAPHIR.

THE HISTOLOGIC CHANGES IN CARCINOMATOUS OSTEOMALACIA. K. MAKRYCOSTAS, Frankfurt. *Ztschr. f. Path.* **40**:501, 1930.

The author reports a case of a primary carcinoma of the uterus in a woman 79 years of age, with metastases to both femurs, both tibiae, fibulae and the metatarses. Special attention is given to the histologic lesions of the bones which are described in greatest detail. Also the synovial changes are carefully studied. The bone and spongiosa were destroyed first, while in some instances the cartilaginous articular end-portions were preserved, loosely attached to the underlying carcinomatous tissue which had completely replaced the osseous portions. Eventually, the cartilaginous end-portions were also replaced by tumor tissue.

O. SAPHIR.

MALIGNANT CYSTADENOMAS OF THE LIVER. I. TOUSSAINT, Frankfurt. *Ztschr. f. Path.* **40**:538, 1930.

Two cases are reported. In both, carcinomatous portions were found in addition to structures of benign adenomas with large spaces justifying the term "cysts." The author believes that only one similar case is reported in the literature. This is the case of R. Jaffe (*Frankfurt. Ztschr. f. Path.* **21**:26, 1918), who found such a tumor in the liver of a dog. It is thought that this tumor arises from bile ducts on the basis of a developmental disturbance.

O. SAPHIR.

CANCER AND LIPOID METABOLISM. F. BURGHEIM and W. JOEL, Klin. Wchnschr. **10**:397, 1931.

The cholesterol content of malignant tumors is always greater than in benign tumors, and there may be a relative increase in cholesterol in the vicinity of cancers. This increase may occur in the precancerous stage. The importance these results may have in the microscopic diagnosis of cancer is emphasized.

COMPLEMENT FIXATION WITH ALCOHOLIC CARCINOMA EXTRACTS. M. FLOKSZTRUMPF and J. KOŁODZIEJSKI, Klin. Wchnschr. **10**:1120, 1931.

The theoretical postulates of the Hirszfeld and Halber carcinoma reaction rest on the following observations: (1) the serums of rabbits immunized with carcinoma extracts react selectively with alcoholic extracts of carcinoma, and (2) the serum of patients with carcinoma binds complement with cholesterolized alcoholic extracts of carcinoma, but not with similar extracts of normal tissues. A total of 475 carcinoma serums and 2,406 control serums were tested. The percentage of positive reactions in carcinoma varied between 0 to 80 according to the origin of the primary growth and the clinical progress of the disease. Early cases of carcinoma were not detected by this method. Visceral carcinoma, especially when disseminated, was detected in 80 per cent, skin carcinomas rarely. In cases of rapidly fatal carcinoma positive reactions rarely occurred. The serums that gave

positive Wassermann reactions and those of pregnant women among the controls reacted positively with the cancer antigen. Patients with hyperthyroidism often reacted positively. Thus 4 to 5 per cent of the control serums reacted positively. These anomalous reactions are without explanation.

EDWIN F. HIRSCH.

### Technical

NEW TECHNIC AND INSTRUMENT FOR OBTAINING BIOPSY SPECIMENS. WILLIAM J. HOFFMAN, *Am. J. Cancer* **15**:212, 1931.

A new technic and instrument are presented for obtaining specimens for biopsy.

B. M. FRIED.

STANDARDIZATION OF METHOD BASED ON MESOBILIRUBINOGEN (H. FISCHER) FOR ESTIMATION OF UROBILINOGEN. C. J. WATSON, *Arch. Int. Med.* **47**:698, 1931.

A modification of Terwen's method for the estimation of urobilinogen in urine and feces is described, which is believed to be simpler and more efficient than the original procedure. The standardization of the color standard is based on crystalline mesobilirubinogen (Fischer). The results of a study of a series of cases with this method are reported. They indicate that valuable information may be obtained by its use in the clinical study of jaundice and the anemias. Persistent urobilinuria after the reticulocyte crisis in pernicious anemia in cases in which liver therapy is used is accompanied by a slower rate of increase of hemoglobin and, to a less marked degree, of erythrocytes.

AUTHOR'S SUMMARY.

SERUM AND PLASMA BILIRUBIN: A COMPARATIVE QUANTITATIVE STUDY OF ONE HUNDRED CASES. MENDEL JACOBI, REUBEN FINKELSTEIN and RUDOLPH KURLIN, *Arch. Int. Med.* **47**:759, 1931.

The results of simultaneous quantitative determinations of serum and plasma bilirubin by the Thannhauser and Andersen modification of the van den Bergh technic in 100 consecutive and unselected cases are reported, and the results are analyzed as to the degree of agreement or disagreement. The readings are shown to correspond in a very high degree, and they indicate that plasma instead of serum may be used with equal accuracy in determinations of bilirubin. The results confirm the statements of McNee and Keefer. They are directly opposed to the figures reported by Shay and Schloss from eleven observations with wide discrepancies in the readings. An increase in the length of time allowed for coupling up to one hour apparently plays no part in the slight quantitative differences noted.

AUTHORS' SUMMARY.

MODIFIED ASCHHEIM-ZONDEK TEST FOR PREGNANCY. F. EBERSON and M. H. SILVERBERG, *J. A. M. A.* **96**:2176, 1931.

The modification consists in injecting into immature female rats of the same litter, from 18 to 21 days old, a suspension of a precipitate obtained by adding alcohol to the urine. By injecting 1 cc. of the test material twice a day for one or two days, the time required to reach a diagnosis has been reduced to from thirty-six to forty-eight hours. When the test is positive, the ovaries, tubes and uterus are enlarged; the tubes are distended, and the ovaries are hemorrhagic and congested, with protruding follicles and "blood points." "In the absence of macroscopic changes in the ovaries, the microscopic observations of a peripheral organization of follicular cells and a peculiar vitreous alteration of the central mass of cells is diagnostic of very early pregnancy or of its arrest. Despite the absence of luteinization, these structural and tinctorial changes noted in the follicular cells

are specific and especially valuable in conditions that preclude diagnosis from the macroscopic observations alone." This method has given uniformly satisfactory results.

NEW LITHIUM SELECTIVE AND ENRICHMENT METHODS FOR THE ISOLATION OF SALMONELLA ORGANISMS. J. D. A. GRAY, *J. Path. & Bact.* **34**:335, 1931.

*Staphylococcus*, *Bacillus foecalis-alkaligenes* and those members of the *Salmonella* group concerned with human disease are markedly resistant to the salts of lithium (chloride and carbonate). For a selective method for the isolation of members of the *Salmonella* group, the addition of 2, 2.5 and 3 cc. of 10 per cent aqueous solution of lithium chloride to plates containing 15 cc. of MacConkey's medium is recommended. Forty-eight hours' incubation is usually required. For an enrichment method for the isolation of members of the *Salmonella* group, the addition of 1, 2 and 3 cc. to 10 cc. of peptone water ( $p_H$  7.6) is recommended. Incubation for twenty-four or for forty-eight hours before subinoculation on MacConkey's medium gives the best results. These selective and enrichment methods were found to give results for the isolation of members of the *Salmonella* group quite as good as, and usually better, than those obtained with the brilliant green enrichment method with or without the addition of telluric acid. Both MacConkey's medium and the peptone water containing lithium chloride may be kept ready for use. Little or no effect on the selective action of the lithium chloride was obtained by the presence of sodium chloride, brilliant green, telluric acid or brilliant green and telluric acid. Removal of the particulate organic matter in the fecal emulsions gave slightly better chances of isolating the *Salmonella* organisms.

AUTHOR'S SUMMARY.

NEW COLLODION MEMBRANES FOR BACTERIOLOGICAL USE. W. J. ELFORD, *J. Path. & Bact.* **34**:505, 1931.

A new series of graded ultrafilter membranes suitable for general bacteriologic use is described. The principles and technic of the preparation of these membranes are discussed in detail. A wide range in permeability is available, from 3 microns down to 10 micromicrons or less. The membranes have excellent uniformity.

AUTHOR'S SUMMARY.

ASBESTOSIS BODIES IN THE FAECES IN PULMONARY ASBESTOSIS. S. ROODHOUSE GLOYNE, *Tubercle* **12**:158, 1931.

As a result of an examination of feces for asbestosis bodies, the author believes this method, in the absence of findings in the sputum, forms an alternative, although admittedly a poor, substitute for clinching the diagnosis.

H. J. CORPER.

QUANTITATIVE DETERMINATION OF ACETONE WITH PULFRICH'S PHOTOMETER. C. URBACH, *Biochem. Ztschr.* **236**:164, 1931.

Urinary acetone is quantitatively determined with Pulfrich's photometer by use of the following technic: From 20 to 100 cc. of a twenty-four hour specimen of urine, kept on ice and well mixed, is put into a distillation flask and the flask filled up to 300 cc. with distilled water. Then 5 cc. of concentrated sulphuric acid is added, and the mixture is distilled into a flask containing 25 cc. of distilled water. After the distillate is filled up to 200 cc., 2 cc. of the following potassium hydroxide solution is added: solution 1, compound of 11.33 normal solution of potassium hydroxide, or 63.6 Gm. of potassium hydroxide in 100 cc. of water, and 1 cc. of salicylic aldehyde. (Solution 2: 10 per cent [Gm.] solution of salicylic aldehyde in 95 per cent alcohol.) The mixture is heated while being shaken over a water bath at 50 C. for exactly twenty minutes, till a red color develops. After

cooling, it is diluted to 15 cc. The distillation is not necessary if the urine does not contain any sugar. Pulfrich's photometer with filter S 53 or S 50 is used, the distillate being compared with a mixture of 10 cc. of solution 1 plus 20 cc. of solution 2 plus 120 cc. of water. After determination of the extinction coefficient, the amount of acetone can be read from a table of predetermined amounts of acetone.

WILHELM C. HUEPER.

#### THE VALUE OF THE PRECIPITATION TESTS FOR THE DIAGNOSIS OF SYPHILIS.

F. E. HAAG and I. LINKWEILER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **70**:337, 1931.

*The Precipitation Test as a Routine Procedure in the Serodiagnosis of Syphilis (I. Linkweiler).*—One thousand serums were tested in the Hygienic Institute in Düsseldorf with three complement-fixation and five precipitation tests: (1) the original Wassermann test (syphilitic liver extract), (2) citochol extract, (3) Bordet extract, (4) the Meinicke test, (5) the Kahn test (original method), (6) the Kahn test with citochol extract, (7) the citochol test (original method) and (8) the Sachs-Georgi test with citochol extract. All tests agreed in 93.5 per cent. Only 1.3 per cent of the cases showed absolute disagreement, the disagreement of the balance being due to qualitative differences. The citochol extract proved particularly sensitive.

*The Practical Significance of Positive Precipitation Tests in the Presence of Negative Complement-Fixation Tests (F. E. Haag).*—The results of six comparative tests on 11,345 serums and spinal fluids are reported. The aforementioned three complement-fixation tests and the following three precipitation tests were used: (4) the Kahn-citochol reaction, (5) the Meinicke test and (6) the Müller-Ballungs test. Agreement was found in 94 per cent and absolute disagreement in 1.1 per cent of the cases. The precipitation tests proved more sensitive in old cases in which treatment had been given. The Kahn test with the citochol antigen showed the highest sensitiveness and specificity. In 460 old cases in which treatment had been given, the complement-fixation was negative 280 times, and precipitation only 14 times.

I. DAVIDSOHN.

#### A METHOD FOR COUNTING DEAD AND LIVING TUBERCLE BACILLI IN THE SPUTUM. E. BUTSCHOWITZ, *Ztschr. f. Tuberk.* **60**:149, 1931.

Tubercle bacilli are counted in smears made from digested sputum to which a known number of yeast cells have been added. The sputum is cultured at the same time. The author believes that by comparing the number of microscopically demonstrable tubercle bacilli with the number of colonies the ratio of dead and living bacilli can be determined.

MAX PINNER.

## Book Reviews

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### A COLLECTIVE REVIEW OF RECENT BOOKS ON CLINICAL PATHOLOGY\*

ARTHUR H. SANFORD, M.D.

ROCHESTER, MINN.

Clinical pathology has developed into a true medical specialty in the last quarter of a century. No greater evidence of this is necessary than the considerable amount of literature that is devoted to this field. If one were to include European literature, there would be no less than thirty-five texts of various kinds that have appeared in the last three years. I shall not attempt, however, to review any books except those that are printed in English. Almost all of these are American books.

Mention might be made first of a new journal in this field, *The American Journal of Clinical Pathology*, which appeared in 1931, published by Williams and Wilkins Company and under the editorship of T. B. Magath. The first volume will number about five hundred pages. The journal is the official publication of the American Society of Clinical Pathologists, a society consisting of specialists in the field, who have devoted more than three years to clinical pathology.

**Manual of Physical and Clinical Diagnosis.** By Dr. Otto Seifert (late Professor of Medicine, Würzburg) and Dr. Friedrich Mueller (Professor of Medicine, II Medical Clinic, Munich). Authorized translation from the twenty-fourth German edition by E. Cowles Andrus, M.D. (Associate in Medicine, Johns Hopkins University; Associate Physician, Johns Hopkins Hospital). Price, \$6.00. Pages, 543 + xi, with 140 illustrations and three colored inserts. Philadelphia and London: J. B. Lippincott Company.

The book of Seifert and Mueller, is not, strictly speaking, on clinical pathology alone, but there is so much laboratory information in it and it is such a useful handbook that it is well worth mentioning. It is in its twenty-fourth edition in German. From this edition, we have the first American translation, by Andrus. The original preparation of this manual in German was prompted by Prof. C. Gerhardt. The data presented, on account of their quantity and diversity, can only with difficulty be accurately memorized. On the other hand, they are distributed in so many textbooks and monographs that it is tedious and time-consuming to hunt them out on each occasion. George E. Fahr of the University of Minnesota, representative of the author, presents an introductory preface in which he says: "Friedrich Mueller needs no recommendation from anyone. The twenty-four editions in the German language and the numerous editions in seven other languages attest to the value of this compendium of diagnostic methods and data. Every interne and clinical clerk should carry a copy in his white coat to be available when working on the wards or in the clinical laboratory. The student who has had a course of lectures on physical diagnosis and on clinical microscopy and chemistry will find that this little book is all that he will usually need to consult in his work on the wards. The practitioner of medicine

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\* Submitted for publication, Oct. 30, 1931.

\* From the Section on Clinical Pathology, the Mayo Clinic.



who has a copy on his desk will only rarely have to consult any other work on diagnostic methods and data." The book may be called a true *vade mecum*. The translator states in the preface that "certain additions have been made to include procedures to which the American student and physician are accustomed: blood chemistry, staining methods, etc."

There are eleven chapters and an appendix. The eleven chapters are arranged as follows: introduction; respiratory organs; circulatory organs; the blood; urinary system; gastro-intestinal tract; parasites and infectious diseases; nervous system; the glands of internal secretion; metabolism and nutrition; diseases of the skin. The appendix contains a tabular summary of the symptoms and the therapy of acute intoxications, mineral springs and baths, drugs and dosage, and troy weights and metric equivalents. There are three colored inserts, one of these being double and presenting excellent illustrations of various types of blood cells. The clinical laboratory material is of course brief as regards actual technic, and the book is not designed as a laboratory manual, but it will be found useful as a reference book for the clinical pathologist in discussing clinical diagnosis with the clinician and will also be informative for the clinician in evaluating the reports of the clinical pathologist. The thin paper edition makes the book convenient in size, so that it can be readily carried in the overcoat pocket or in the physician's handbag.

**Bedside Interpretation of Laboratory Findings.** By Michael G. Wohl, M.D. (Associate Professor of Experimental Medicine, Temple University Medical School; Chief of the Metabolic Clinic, Temple University Hospital; Chief of the First Medical Diagnostic Clinic, Mount Sinai Hospital, Philadelphia, Pa.). With an introduction by Joseph McFarland, M.D., Sc.D. (Professor of Pathology, University of Pennsylvania). Price, \$6.00. Pages, 321, illustrated. St. Louis: The C. V. Mosby Company, 1931.

Wohl states in his preface that the purpose of the volume is to deal with the middle ground between the fields of activity of the clinical pathologist and the general practitioner. Simple tests that can be carried out by the practitioner in his office are given in detail. The proper evaluation of the more complicated tests carried out by the clinical pathologist is presented for the use of the clinician in making the diagnosis. The introduction by McFarland contains some timely comments on the present-day physician's knowledge of laboratory procedures and his attitude toward "reports."

The twenty-three chapters of the text deal with the clinical interpretation of various tests under the following headings: sputum; gastric contents; duodenal contents; feces; blood; blood chemistry; blood cultures; blood groupings; acidosis and alkalosis; basal metabolism; urine; kidney functional tests; liver functional tests; cerebrospinal fluids; transudates and exudates; immunologic reactions; skin reactions; mouth and pharynx; eye and ear; skin (really a chapter on mycology); infections of the urethra and external genitalia; laboratory tests for pregnancy; miscellaneous tests. It will be seen from the diversity of subjects that the euphonious title of the book is somewhat of a misnomer, as in a large proportion of instances the interpretation of the laboratory results are not made at the bedside. The clinician, however, by using this book in his study, may gain the necessary information for an intelligent discussion of his case with the clinical pathologist, who should act as a consultant. On the other hand, the clinical pathologist should find this book a valuable adjunct in such consultations by using the concise information that is here given concerning the clinical interpretation of laboratory data. The selection of material has been made by the author from his extensive and valuable experience as a clinical pathologist. The chapter on laboratory tests for pregnancy by Mazer and Hoffman is of interest because of the description of the test for the female sex hormone originally described by these authors, using spayed mice as the test animals. It is regrettable, however, that mention is not made of the simple Friedman test, which is rapidly gaining in favor. This book should make good reading for the busy physician who wishes to keep up to date on diagnostic methods.

**The Clinical Interpretation of Blood Examinations.** By Robert A. Kilduffe, A.M., M.D. (Director of Laboratories, Atlantic City Hospital, and City Bacteriologist, Atlantic City, N. J.). Price, \$6.50. Pages, 629, illustrated. Philadelphia: Lea & Febiger, 1931.

This is a more pretentious book than the one just reviewed, but in a narrower field. Kilduffe has demonstrated on many occasions his ability to state clearly the technic employed in various clinical laboratory procedures and the interpretations of the results. This new volume is an expansion of a previous compendium entitled "The Clinical Interpretation of the Wassermann Reaction." This volume contains thirteen chapters. The first chapter, on the physical properties of the blood, is clearly and ably written. This is followed by chapters on: blood formation and blood destruction; cytologic blood examination; clinical study of the cellular elements of the blood; the leukocytes; the blood platelets; the symptomatic blood picture in disease; laboratory studies of anemias; bacteriologic examination of the blood; serologic examination of the blood; the parasitology of the blood; the complement-fixation test; chemical examination of the blood. There are numerous references to the literature in footnotes, and the book will undoubtedly furnish a valuable guide to both the clinician and the laboratory worker in further study on the immense number of subjects that are treated. The technic is not given in detail, except in some of the simple procedures; it is not intended to be a laboratory manual. Probably the best chapter is the twelfth, on complement-fixation tests. In this field the author is well known, and he has put more of his personal experience into the writing of this chapter. In the final chapter, the subject of blood chemistry is as adequately handled as can be expected in the space allotted, about 125 pages. There is little to criticize unless it is that the author has included many unnecessary references to valueless tests. The author states in his preface that he has done this deliberately, feeling that it is due to the reader to have an evaluation put on the unsatisfactory, as well as on the valuable, diagnostic procedures. However, in reviewing such a collection, the reader becomes weary of so much that is "non-specific" or of "little value." This is especially noticeable in the tenth chapter, in which ten pages, including comment on more than ten tests, are given to serologic studies in malignancy, all of no clinical value. In the same chapter, seven pages are devoted to unsatisfactory laboratory tests for the diagnosis of pregnancy, although every clinical pathologist is more or less familiar with the satisfactory Aschheim-Zondek test or the simple Friedman test. One wonders, in the consideration of agglutination tests, why Maniow's test for the determination of sex is included, whereas the useful agglutination tests for diagnosing undulant fever are omitted, and only one and a half lines are devoted to the subject of tularemia. In the chapter on the symptomatic blood picture in disease, the various disorders are arranged alphabetically. Such an arrangement is natural enough, although it is somewhat disconcerting to find cholera, fractures, hay fever and heart disease considered in the order named. Although these minor criticisms are mentioned, on the whole the book will undoubtedly be found useful in the library of every clinician who has not been able to keep abreast of the vast amount of work that has been done in recent years in the field of examinations of the blood.

**Quantitative Clinical Chemistry.** By John P. Peters, M.D., M.A. (Professor of Internal Medicine, Yale University School of Medicine) and Donald D. Van Slyke, Ph.D., Sc.D. (Member of the Rockefeller Institute for Medical Research). Vol. I: Interpretations. Price, \$12.00. Pages, 1264 + xvi. Baltimore: Williams & Wilkins Company, 1931.

Peters and Van Slyke's book on interpretations of laboratory procedures will doubtless last for many years as the best example of such writing. The first volume has just appeared and bears a subtitle "Interpretations." These authors have prepared a monumental work. If the second volume, which is to be on technic, is as complete as this volume on interpretations, it is safe to say that it will be the authoritative work in English on the subject of clinical chemistry.

There are 3,890 references to the literature in this volume alone, which make it exceedingly valuable if considered only for this useful feature. The style is clear and readable for the clinician or laboratory worker who is not entirely conversant with all of the technical procedures considered. The reputation of the authors in the scientific world for exact evaluation of scientific data is upheld in every particular. The chapters are on: total metabolism; carbohydrates; lipids; nonprotein nitrogen and nitrogen metabolism; urine, ammonia; amino-acids; uric acid; creatine and creatinine; total organic acids; lactic acids and ketones; phenols; hemoglobin; proteins of the blood plasma, urine and other body fluids; blood volume; total base, sodium and potassium; calcium, magnesium; carbonic acid and acid-base balance; chlorides, phosphorus and sulphur. We shall await with much interest the appearance of the second volume.

**Chemical Methods in Clinical Medicine.** Their Application and Interpretation with the Technique of the Simpler Tests. By G. A. Harrison, B.A., M.D., B.Ch. (Cantab.), M.R.C.S. (Eng.), L.R.C.P. (Lond.) (Reader in Chemical Pathology, University of London; Reader and Lecturer on Chemical Pathology, St. Bartholomew's Medical College; Chemical Pathologist, St. Bartholomew's Hospital; Formerly Chemical Pathologist, King's College Hospital and Hospital for Sick Children, Great Ormond St., London). Price, \$5.25. Pages, 534 + ix, with two color plates and 63 illustrations. New York: The Macmillan Company, 1930.

Harrison's book was printed in Great Britain, but was distributed in this country by the Macmillan Company. It contains twenty-six chapters, an appendix and a well arranged index. The introductory chapters are: definition of terms; preparation of standard solutions; description of apparatus. The next fifteen chapters are on urinalysis; in fact, the author makes much over the point that unfortunately in recent years interest has waned in this important field of clinical chemistry owing to the overshadowing attention that has been paid to chemistry of the blood. Some of the chapters, to be sure, are brief. They are listed as follows: routine qualitative tests; albuminuria, both qualitative and quantitative tests; urinary deposits; tests of renal efficiency, with a very good, detailed description of the Maclean-de Wesselow test (urea-concentration test); reducing substances in the urine, blood sugar curves; chemical tests in diabetes mellitus and the control of insulin treatment; ketosis, acidosis and alkalosis, clinical significance, and technical methods; blood and its derivatives in the urine, the direct vision spectroscope; urine abnormal in color, with special reference to drugs in the urine; bile and urobilin in the urine, with efficiency tests of the liver and bile passages; tests of pancreatic efficiency; indicanuria, lipuria and skatole-red, diazo-test, chyluria, nitrituria; chlorides, and inorganic constituents of the urine; the collection, preservation and quantitative analysis of the urine. There are three chapters on blood: the collection and preservation of blood; qualitative examination, in which the "formol-gel test" and Jaffe's test for indicanemia are considered; chemical analysis of the blood. The last seven chapters are devoted to: chemical examination of the cerebrospinal fluid; analysis of milk; gastric analysis; chemical examination of duodenal contents; chemical examination of feces; basal metabolism and metabolism experiments; "miscellanea and conundrums." The last chapter tells among other things how to determine whether the urine is contaminated with feces or whether a laparotomy wound contains pancreatic juice. As these and similar questions occasionally tax the ingenuity of the clinical pathologist, the author's solutions make interesting reading. The appendix also contains a number of useful tables and miscellaneous information.

Harrison pays particular attention to calculations. Seldom is each step in the mathematic computation of a test elaborated in such detail. This is deliberately done by the author, as he stated in the preface: "That the calculations are given very fully—some readers may consider in absurd detail. . . . In the author's experience more mistakes are made in the calculations than in the actual technic by the average student and technical assistant and also because in his opinion many writers give calculations too briefly."

For the most part this book will prove interesting and instructive to American readers. The simple illustrations of the line-drawing type are useful in clarifying technical procedures. One criticism may be made of the manner of making cross-references. The reference is always merely to a chapter and not to the page or pages. The index, however, is complete and well arranged. There are also complete references to other texts and to the literature generally with each chapter.

**Clinical Laboratory Methods.** By Russell Landram Haden, M.A., M.D. (Professor of Experimental Medicine, University of Kansas School of Medicine, Kansas City, Kan.). Edition 3. Price, \$6.75. Pages, 317, with 69 illustrations and four color plates. St. Louis: The C. V. Mosby Company, 1929.

**Practical Clinical Laboratory Diagnosis Including the Interpretation of Laboratory Findings.** Designed for the Use of Students and Practitioners of Medicine. By Charles C. Bass, M.D. (Dean and Professor of Experimental Medicine, School of Medicine, Tulane University of Louisiana) and Foster M. Johns, M.D. (Assistant Professor of Medicine and Director of the Laboratories of Clinical Medicine, the School of Medicine, Tulane University of Louisiana) Edition 3. Price, \$7.50. Pages, 187 + xvi, with 134 black and white textual figures and 20 plates, nine of which are in color. Baltimore: Williams & Wilkins Company, 1929.

**A Manual of Clinical Laboratory Methods.** By Clyde Lottridge Cummer, Ph.B., M.D., F.A.C.P. (formerly Associate Clinical Professor of Clinical Pathology, School of Medicine, Western Reserve University, Cleveland; Instructor in Dermatology and Syphilology, School of Medicine, Western Reserve University; Visiting Dermatologist, Charity and St. Alexis Hospitals, Cleveland). Edition 3, thoroughly revised. Price, \$6.75. Pages, 583 + xx, with 173 engravings and 12 plates. Philadelphia: Lea & Febiger, 1931.

**Clinical Diagnosis by Laboratory Methods.** A Working Manual of Clinical Pathology. By James Campbell Todd, Ph.B., M.D. (late Professor of Clinical Pathology, University of Colorado, School of Medicine) and Arthur Hawley Sanford, A.M., M.D. (Professor of Clinical Pathology, University of Minnesota [Mayo Foundation]; Head of Section on Clinical Laboratories, Mayo Clinic, Rochester, Minn.) edition 7, thoroughly revised. Price, \$6.00. Pages, 765, with 347 illustrations, 29 in colors. Philadelphia: W. B. Saunders Company, 1931.

Within the last three years there have been new editions of these four of the leading texts. Although it may not seem necessary to give a complete description of each of these well known books, it would seem best to comment on them in this review, since the scope of each one of these works may not be known to all readers.

Haden's book appeared in its third edition in 1929. The book was thoroughly revised and brought up to date. It is not a textbook; the author has rather presented his notes in the form that he has found useful in the laboratory of a general hospital. His wide experience in medicine and in teaching has enabled him to make a useful selection of those diagnostic procedures that he believes are most worthwhile. There is no attempt to explain results. Normal values are often given, however. In fact, among the chief features of the book are several tables in which normal and abnormal data are listed. One of the most interesting of these is the first table in the book on the identification of reducing substances in the urine. By carrying out half a dozen tests in the order listed, various reducing substances, as well as dextrose, may be identified. Another convenient and unique feature of the book is the placing of the reference to the original article immediately below the title for the text. There is no hunting in a bibliography at the end of the book or even in a footnote for the reference to the literature.

There are fourteen chapters, arranged as follows: qualitative examination of urine; quantitative chemical examination of urine; analysis of gastric juice; examination of sputum; examination of feces; qualitative examination of the blood; quantitative chemical examination of the blood; serologic technic; preparation of bacteriologic solutions; stains and mediums; general bacteriologic methods; miscellaneous clinical pathologic examinations; miscellaneous chemical procedures and solutions; histologic technic; examination of milk and water. A thoughtful study of the numerous tables and the brief descriptions of the various tests will commend this book to the laboratory worker and clinician alike. The diction is clear and concise. The nomenclature in the consideration of animal parasites and of bacteria is correct and accepted.

In 1929, also, appeared the third edition of Bass and Johns' book. This small book contains much interesting and original matter. It is chiefly a "picture book." The authors have evidently felt that accurate photographs illustrating the various steps of technical procedures would impress the student more readily than a word description. There are 134 of these illustrations in black and white, and twenty plates, nine of which are in colors, scattered through 179 pages of text. Some of these illustrations are well done, while others would seem to be unnecessary. They may emphasize certain laboratory rules for the medical student, but have no real part in the practice of clinical pathology by an experienced worker. The authors have a somewhat original method of arranging their text. There are seventeen chapters, many of them arranged in a manner found in most other books, but some of the chapters deal with only a single disease and the tests connected with the diagnosis of that condition. This is not strange when one remembers the interesting pathologic material in certain fields in New Orleans. The first five chapters are: use and care of the microscope; blood smears and stains; differential leukocyte counts; total counts in hemoglobin estimations; special tests of blood. The latter includes the determination of blood sugar, which is the only test having to do with the chemistry of the blood described in the book. The sixth chapter is brief and deals solely with malaria. Then follows chapters as follows: typhoid agglutination tests; urine; gastric contents; feces; pus, exudates and similar material; sputum. The chapter on feces is doubtless one of the best chapters in the book. The thirteenth chapter is one and a half pages in length and is on the laboratory diagnosis of leprosy. The last four chapters are on: spinal fluid; diphtheria; gonorrhea; syphilis. None of the various modifications of the Wassermann test is described, and the only serologic test that is given in detail, and that rather briefly, is the Butler precipitation test. Although this may be an excellent test, it is not as well known as the Kahn or the Kline test, of which no mention is made. Two appendixes contain the usual formulas and lists of recommended apparatus found in most textbooks.

The third edition of Cummer's well known manual appeared in 1931. The book is brought up to date in the twelve chapters and appendix, which are arranged as follows: examination of the blood; pathologic histology and differential diagnosis; parasitology and bacteriology of the blood; immunology; examination of chemical changes in the blood; urine; gastric and duodenal contents; feces; sputum; body fluids; basal metabolism; miscellaneous methods. The eleventh chapter, on basal metabolism, by M. A. Blankenhorn, includes a description of the Benedict method. The twelfth chapter is on bacteriologic methods. The appendix contains some valuable miscellaneous information. A scheme for the examination of a large number of specimens of urine in a hospital laboratory is described in considerable detail. The preparation of normal solutions is well presented. There are also a dozen similar subjects that reflect the experience of the author and that will appeal to the reader. A rather unique feature is the printing on the back fly-leaf and inside cover of a table of normal findings. However, it is unfortunate that the bibliography at the end of the book has not been brought up to date; this is particularly true of the textbooks. The reader is entitled to references to the latest editions. In many instances, several editions have appeared since the one that is referred to.

Probably the best chapter in the book is the fourth, on immunology. In the consideration of agglutination tests, both tularemia and undulant fever are described. The Kolmer-Wassermann technic is given fully, also a most interesting and complete description of the so-called Cleveland method, which was developed by a committee of various laboratory workers appointed by the Academy of Medicine of Cleveland, in the hope that a uniform technic would thereafter be used in Cleveland. Thus the test was named after the city in which it was developed. Both the Kline and the Kahn flocculation tests are given satisfactorily. Criticism must be made of the chapter on intestinal parasites. The choice of *Dibothrycocephalus latus* for the name of the broad Russian tapeworm is not now accepted by parasitologists, who prefer, on good and sufficient grounds, *Diphyllobothrium latum*. One is also somewhat amazed to read in the consideration of "examination for amoeba" that "the examination should be conducted at the bedside when possible." Is this ever done? The book is well illustrated and indexed, and the author is to be congratulated on this new edition, which includes much new material: for example, the silver impregnation methods for spirochetes, the Alzheimer method for studying the cellular contents of the spinal fluid, a description of cistern puncture, the alcohol test meal and the use of histamine in gastric analysis.

This year (1931) has also seen the appearance of the seventh edition of Todd and Sanford's book. This book probably needs no extensive review. The original author, the late Dr. Todd, was well known as a master of his subject. The joint author, to whom has fallen the task of bringing out the present and future editions, has endeavored to bring the book up to date. A few of the little used or obsolete tests have been omitted. The new material includes Corper and Uyei's method for the culture of bacteria of tuberculosis, Fairhall's method for the determination of lead, Folin's method (1929) for the precipitation of protein from blood and body fluids, his modified method for the determination of uric acid in the blood, and his revised copper solution for the determination of blood agar. Clark and Collip's method for the determination of calcium is given in full. The technic of the Keith, Rowntree and Geraghty method of determining blood volume and plasma volume is given in detail. The alcohol meal and the gastric reaction to histamine are considered in the chapter on analysis of gastric contents. The Gregerson test for occult blood is included. The Aschheim-Zondek test and the simpler Friedman test for pregnancy are briefly described. The chapter headings are: sputum; urine; blood; gastric and duodenal contents; feces; animal parasites; pus; puncture fluids, animal inoculation; miscellaneous examinations serodiagnostic methods; bacteriologic methods; vaccines and biologic skin tests. The paper used is thin, so that the book is larger than it appears, there being 765 pages. Some of the plates are becoming worn, and it is hoped that in future editions more attention may be paid to the illustrations.

**Laboratory Medicine.** A Guide for Students and Practitioners. By Daniel Nicholson, M.D. (Member of the Royal College of Physicians, London; Assistant Professor of Pathology, University of Manitoba; Assistant in Pathology, Winnipeg General Hospital). Price, \$6.00. Pages, 437, with 108 engravings and a colored plate. Philadelphia: Lea & Febiger, 1930.

Nicholson's book has been thoroughly reviewed in *THE ARCHIVES* (11:168, 1931). The author has put into this his own experiences as a clinical pathologist. The result is pleasing, and the book has been well received.

**A Textbook of Laboratory Diagnosis with Clinical Applications for Practitioners and Students.** By Edwin E. Osgood, M.A., M.D. (Assistant Professor of Medicine and Biochemistry, and Director of Laboratories, University of Oregon School of Medicine) and Howard D. Haskins, M.D. (Professor of Biochemistry, University of Oregon School of Medicine, Portland, Ore.). Price, \$5.00. Pages, 475 + xix, with 21 figures and 6 colored plates. Philadelphia: P. Blakiston's Son & Company, 1931.

Osgood and Haskins embody, in pleasing form, their lecture notes as presented to their students. The authors are both well known, particularly in the

field of hematology and chemistry of the blood. Their book is divided into two parts. Part I is written from the point of view of the general practitioner, with the clinical value and the interpretation of certain laboratory procedures considered more or less fully in relation to various diseases and symptoms. The arrangement is original but not entirely logical. Disorders of the kidney and urinary tract with special reference to nephritis are treated first, and are naturally followed by a chapter on disorders of carbohydrate, protein and fat metabolism with special reference to diabetes mellitus and disturbances of acid-base equilibrium. Pregnancy and its complications next claim attention, followed by disorders of the central nervous system with emphasis on the differential diagnosis of coma. The remaining four chapters of part I are on: disorders of the gastro-intestinal tract; basal metabolism; hematology; respiratory and cardiac disorders.

Part II takes up fully the technical procedures in common use referred to in part I. The arrangement is satisfactory enough if the reader follows the suggestion that "this text is to be thought over thoroughly, not merely to be read or memorized by rote." The numerous references to the literature are carefully selected and should stimulate the student to much collateral reading. He will also find useful the well planned index of diseases, which is, however, "not a substitution for cerebration." The chemical procedures, as might be expected, are well presented. There are many modifications of well known tests that are introduced from the experience of the authors.

Not enough attention is paid to examination of the feces; only eight pages with one table and one plate in which the illustrations are not adequate are devoted to the subject. This is probably the weakest part of the book. The Aschheim-Zondek test for pregnancy is well presented, but no mention is made of the simpler Friedman test, which is probably as reliable. Hematology is considered in an interesting, instructive and stimulating manner. It is safe to say that the color plates, reproduced from the drawings by Miss Clarice Ashworth, are as fine as any illustrations of blood cells that have appeared in American publications. The printing as a whole and the general appearance of this first edition lead one heartily to congratulate the authors.

**Approved Laboratory Technic, Clinical, Pathological, Bacteriological, Serological, Biochemical, Histological.** Prepared under the auspices of The American Society of Clinical Pathologists. By John A. Kolmer, M.D., Dr. P.H., D.Sc., LL.D. (Professor of Pathology and Bacteriology, Graduate School of Medicine, University of Pennsylvania; Professor of Immunology and Chemotherapy, School of Medicine, Temple University; Head of the Department of Pathology and Bacteriology, Research Institute of Cutaneous Medicine) and Fred. Boerner, V.M.D. (Associate Professor of Bacteriology, Graduate School of Medicine, University of Pennsylvania), assisted by C. Zent Garber, A.B., M.D. (Associate in Pathology, Peking Union Medical College; formerly Associate Pathologist, Henry Ford Hospital, Detroit) and by committees of the American Society of Clinical Pathologists, composed of Dr. J. H. Black, Dr. H. J. Corper, Dr. A. G. Foord, Dr. A. S. Giardano, Dr. F. W. Hartman, Dr. P. Hillkowitz, Dr. R. A. Keilty, Dr. R. A. Kilduffe, Dr. K. M. Lynch, Dr. A. H. Sanford and Dr. F. E. Sondern. Price, \$7.50. Pages, 663 + xxii, with 11 colored plates and 300 illustrations in the text. New York and London: D. Appleton & Company, 1931.

This book is one of the most pretentious books on laboratory technic that has been published in America. It was prepared under the auspices of the American Society of Clinical Pathologists. The authors were assisted in this work by Garber and committees of the society. The book is dedicated to Dr. George H. Meeker of the University of Pennsylvania and to the memory of Ward Burdick, founder and first secretary of the American Society of Clinical Pathologists.

The object is "to establish standards for the performance of various laboratory examinations, to promote the practice of medicine by a wider application of clinical laboratory methods to the diagnosis of disease and to encourage a closer coöperation between the practitioner and the clinical pathologist."

The technic of each method has been definitely approved by at least five members of the society, although it cannot be stated that the society as a whole has approved of the methods described. The importance of using accurate and reliable apparatus and reagents is stressed. Quantitative tests are given special emphasis whenever possible. There is also included a brief chapter on methods for the microscopic examination of tissues by W. C. MacCarty and W. L. A. Wellbrock.

The book is divided into five sections and contains thirty-seven chapters, several of them on unusual subjects. The sections are: general laboratory methods; clinical pathologic methods; bacteriologic methods; serologic methods; clinical methods.

The chapters in section I are: use and care of the microscope and methods of micrometry; housing, feeding, inoculating, bleeding and necropsy of animals, and diagnosis of animal diseases; suggestions for routine and special laboratory examinations; prevention and emergency treatment of laboratory accidents.

Section II contains chapters of the more usual descriptions of clinical laboratory procedures. There is a comprehensive chapter on examination of the blood, and chapters on: methods for the examination of urine; renal functional tests; sputum; gastric contents; bile and duodenal contents; tests of hepatic function; feces; exudates and transudates; cerebrospinal fluid.

Section III, on bacteriologic methods, contains nine chapters: methods for the collecting and handling of material for bacteriologic examination; preparation and sterilization of glassware; preparation of culture mediums; general bacteriologic methods; diagnostic bacteriologic methods; preparation of bacterial vaccines; bacteriologic examination of milk; bacteriologic examination of water; testing of disinfectants.

Section IV, on serologic methods, has five chapters: collection of blood and serum; agglutination tests; blood transfusion tests; complement-fixation tests for syphilis and bacterial diseases; precipitation tests for syphilis.

Section V is headed "Clinical Methods." I believe this is a typographical error, as certainly seven of the nine chapters in the section are on chemical methods. There are adequate descriptions of methods of colorimetry, nephelometry and scopometry; preparation of standard solutions; chemical examination of the blood; chemical examination of urine; determination of basal metabolic rate; chemical examination of milk and other foods; methods for toxicologic examination. The thirty-sixth chapter is on the diagnosis of early pregnancy, and the last chapter in the book is on the microscopic examination of tissues. There is a carefully prepared index.

The quality of the paper has added greatly to the beauty of the book, although it has doubtless added greatly to the bulk and somewhat to the price. As a result, the illustrations, which are practically all also "approved," and which are borrowed from other publications, stand out with a remarkable clarity often not found in their original presentation. Plate I represents blood cells stained with "Wilson's stain." This stain is not well known to hematologists, and unfortunately no reference can be found in the text to its use. In collecting material from so many other texts, the authors have committed a not uncommon error in references to the literature: the original authority is not always given correctly. For example, in the description of Wright's stain it is stated that "employing a buffer solution is also recommended by Giardano." Although it is true that Giardano



makes the recommendation, this solution was described several years ago by Haden and in his book credited to McJunkin.

The chapter on methods for the examination of feces contains much valuable information. However, criticism may be made of the use of capitals in the species names of the parasites. Also in the use of *Dibothryocephalus latus* instead of *Diphylobothrium latum* as the name of the "fish tapeworm." All of these are minor faults that may creep into any first edition. The beautiful appearance of the book itself and the wealth of information that it contains should make this a popular reference work for the clinical pathologist.

**Protozoan Parasites of the Alimentary Tract.** By Kenneth M. Lynch, M.D. (Professor of Pathology, Medical College of the State of South Carolina, Charleston, S. C.). Price, \$3.75. Pages, 258 + xvii. New York: The Macmillan Company, 1930.

Although not strictly speaking a work on clinical pathology, mention should be made of Lynch's monograph. The author, a well known pathologist, is perhaps best known for his many publications on protozoology, and especially for his studies of the genus *Trichomonas*. He stated in his preface, however, that the book "is not intended for protozoologists. To biologists it will be an unconventional book. It is purposely so. Technical details which tend to confuse and so lose the interest of those to whom they are of no concern are largely left out of consideration." And in the introduction is the following paragraph: "Protozoologists are not pathologists; few pathologists and other physicians know much about protozoa. Therefore, the errors common among protozoologists in evaluating disease when associated with the presence of protozoa and among physicians in estimating the significance of the finding of protozoa in a sick person." There are fourteen chapters. The first chapter is on the life of protozoa, and the second on dissemination and prevention of infection. Then follow five chapters on the amebas, and these in turn by four chapters on the flagellates. The three remaining chapters are on the ciliates, the coccidia and the blastocystis. Although the book shows evidence of rather hasty preparation, it is an excellent compilation of the author's own views on the subject and should be very useful to the clinical pathologist.

#### COMMENT

It has been necessary to outline somewhat tediously the contents of each of these new publications. However, it will be seen that no one of them can completely cover the field. Although there is, of course, much duplication of descriptions of more or less standard procedures, there is an interesting variety in the manner of presentation, so that in many laboratories and in all large libraries there will be found everyday use for all of these new books on clinical pathology.

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**La Culture des tissus en biologie expérimentale.** By Émile C. Craciun, Maître de conférences à la Faculté de médecine de Bucarest. Preface du Professeur G. Roussy. Price, 55 francs. Pp. 442, with 72 illustrations. Paris: Masson & Cie, 1931.

The reader of this book is left with the impression that a method reputed to be technically very difficult and accessible only to a chosen few was absolved from its former exclusiveness and made available to the mere mortals of the species of laboratory investigators.

The thoroughness and lucidity of presentation and the emphasis laid on minute details, so important in this field, deserve commendation. It will help to gain friends among those laboratory workers who are deterred from approaching this method of investigation by the exaggerated conception of its technical difficulties. Besides the sixty-four pages given to the discussion of general technical details, numerous additional specialized technical procedures are found all through the book. All varieties and modifications of technic are given in a convincingly objective manner, with a recommendation of those that proved best in the author's experience.

The study of tissues *in vitro* became an indispensable method of research for investigators in various disciplines. The general biologist, the zoologist, the botanist, the cytologist, the physiologist, the pharmacologist, the experimental radiologist, the pathologist, the bacteriologist and the immunologist use the technic with great profit, and all are undoubtedly interested in the results achieved with the tissue culture method in their respective fields. These achievements are presented in chapters 2 to 10, with exhaustive references to the original publications. The bibliography of fifty-seven pages appears to be complete.

The value of this excellent book could have been enhanced by the use of a paper of better quality and by good binding. As it is now, it will show quickly the wear and tear, particularly since, by its very nature, it is destined to be used on the laboratory table.

In the reviewer's opinion, the book merits translation to make it accessible to those who do not read French.

## Books Received

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A MANUAL OF CLINICAL LABORATORY METHODS. 'By Clyde Lottridge Cummer, Ph.B., M.D., F.A.C.P., Instructor in Dermatology and Syphilology, School of Medicine, Western Reserve University. Third edition. Cloth. Price, \$6.75. Pp. 585, with 185 illustrations. Philadelphia: Lea & Febiger, 1931.

BULLETIN OF THE NATIONAL RESEARCH COUNCIL No. 83. A COMPENDIUM OF THE STATUTE LAW OF CORONERS AND MEDICAL EXAMINERS IN THE UNITED STATES. By George H. Weinmann. Issued under the Auspices of the Committee on Medicolegal Problems National Research Council. Price, \$3. Pp. 240. Washington, D. C.: The National Research Council of the National Academy of Sciences, 1931.

TRAVAUX DES INSTITUTS D'ANATOMIE PATHOLOGIQUE DES UNIVERSITÉS DE POLOGNE. Editors, S. Ciechanowski (Kraków), W. Nowicki (Lwów), K. Opoczyński (Wilno), L. Paszkiewicz (Warszawa) and L. Skubiszewski (Poznan). Volume 2, nos. 3 and 4. 1931.

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH. HISTORY, ORGANIZATION, PRESENT SCOPE OF THE SCIENTIFIC WORK, BUILDINGS AND EQUIPMENT, PUBLICATIONS. Pp. 36. New York City: 1931.

PROCEEDINGS OF THE PATHOLOGICAL SOCIETY OF PHILADELPHIA. New series, volume 30; old series, volume 48. Containing the Transactions of the Society from January, 1927, to January, 1931. Philadelphia: Isolde T. Zeckwer, M.D., Recorder of the Society, 1931.

ANATOMIE PATHOLOGIQUE. Par Maurice Letulle, professeur honoraire à la Faculté de Médecine de Paris. Avec la collaboration de L. Nattan-Larrier, professeur au Collège de France, et A. Jacquelin, médecin des Hôpitaux de Paris, L. Duclos, ophthalmologiste adjoint de l'Hôpital Chirurgical Gouin, E.-P. Normand, conservateur du musée d'anatomie pathologique générale Maurice-Letulle (Hôpital Boucicaut). Tomes I, II et III. Cloth. Price, 600 francs per set. Pp. 2346, with 843 illustrations. Paris: Masson & Cie, 1931.

STUDIES OF PROTECTION AGAINST TUBERCULOSIS: RESULTS WITH B. C. G. VACCINE IN MONKEYS. By A. Stanley Griffith. Medical Research Council, Special Report Series, No. 152. Paper. Price, 9 pence. Pp. 49. London: His Majesty's Stationery Office, 1931.

REPORT OF THE NATIONAL RESEARCH COUNCIL FOR THE YEAR JULY 1, 1929-JUNE 30, 1930. Established in 1916 at the request of the President of the United States under the charter of the National Academy of Sciences. Paper. Price, 15 cents. Pp. 119. Washington, D. C.: Supt. of Doc., Government Printing Office, 1931.

RESISTANCE TO INFECTIOUS DISEASES: AN EXPOSITION OF THE BIOLOGICAL PHENOMENA UNDERLYING THE OCCURRENCE OF INFECTION AND THE RECOVERY OF THE ANIMAL BODY FROM INFECTIOUS DISEASE, WITH A CONSIDERATION OF THE PRINCIPLES UNDERLYING SPECIFIC DIAGNOSIS AND THERAPEUTIC MEASURES. By Hans Zinsser, M.D., Professor of Bacteriology and Immunology, Medical School, Harvard University. Fourth edition. Cloth. Price, \$7. Pp. 651, with illustrations. New York: The Macmillan Company, 1931.

SURGICAL PATHOLOGY OF THE DISEASES OF BONES. By Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kan., Professor of Surgery, University of Kansas. Pp. 272, with 211 illustrations. Philadelphia: J. B. Lippincott Company, 1931.

## HEMORRHAGE AND "SHOCK" IN TRAUMATIZED LIMBS

CHANGES IN TOTAL, FREE AND BOUND WATER OF BLOOD  
AND MUSCLE \*

WILLIAM ROBINSON, PH.D.

AND

ELOISE PARSONS, M.D.

CHICAGO

The low blood pressure that occurs after hemorrhage and during shock is accompanied, if not directly caused, by a reduction in the volume of the circulating blood. Since blood is a liquid of which from about 78 to 81 per cent is water, it was thought that a study of the changes in the amount and condition of the water present might be another means of investigating the cause of shock. Low blood pressure was produced experimentally in barbital-anesthetized dogs by means of direct hemorrhage, injections of histamine and blows on the leg. The study of the water of the blood includes determinations of total water, and also of what are called "free" and "bound" water.

The dissolved materials and the colloids that are present in the water of the tissues have the effect of modifying some of the properties of the water in which they occur. As a consequence, the water of the tissues does not behave in all respects like water in its pure state, and it is commonly spoken of as "bound" water.

Any variations in the concentrations of substances in solution and any changes in the water-binding capacity of the colloids that take place as a result of normal physiologic or of pathologic processes may affect the degree of force with which the water is bound. Thus there is the possibility (Gortner,<sup>1</sup>) that a shifting takes place, either backward or forward, in the degree of water-binding, and that this may have some bearing on biologic processes.

The conception of some of the earlier workers was that part of the water in the tissues is bound and that the remainder is free; but according to more recent theories, all the water in the tissues is bound, and

\* Submitted for publication, June 13, 1931.

\* From the Otho S. A. Sprague Memorial Institute, the Department of Pathology and the Douglas Smith Foundation for Medical Research, University of Chicago.

1. Gortner, R. A.: *Outlines of Biochemistry*, New York, John Wiley & Sons, 1929.

changing conditions affect only the force with which it is bound.<sup>2</sup> The method employed to determine bound and free water in the present experiments, however, is applicable under either of these two conceptions.

#### METHODS

*Determinations of Bound and Free Water.*—The fact is recognized that the colloids and dissolved materials in water hold it with tenacity, resisting any force that tends to pull the water away. In the present method of making determinations a definite and constant desiccating force is used that tends to withdraw the water. The more firmly the water is bound the less, of course, can be removed. That part that can be withdrawn under a standard desiccating force is called free and the remainder, which is retained, is termed bound water under these conditions. This is an arbitrary distinction, but so long as the dehydrating force is constant this distinction serves in any series of comparative tests.

The method of Rubner,<sup>3</sup> termed the "heat of fusion of ice" method, is used. It consists in placing a known amount of specimen, usually about 0.5 Gm., in a prepared tinfoil container of known weight, freezing the material at a constant temperature of  $-20^{\circ}\text{C}$ . for several hours, transferring the specimen and container to a calorimeter where a determination is made of the number of calories of heat required to melt the ice formed within the tissues. This determination is based on the fact that to melt 1 Gm. of ice without raising its temperature requires 80 calories of heat. By calculation, the amount of freezable water per gram of solid is determined. The final step in the process is to dry the material to constant weight for total water content. The difference between the two values indicates the amount of bound water in the specimen.

In the freezing process an efficient desiccating force is provided, since at temperatures below zero water tends to leave the colloids and dissolved materials to form ice crystals of pure water.

A detailed description of this method has been given by one of us (Dr. Robinson<sup>4</sup>). The process is applicable to both liquids and solids.

*Care of the Dogs.*—In the early experiments, the dogs were used as received from stock. Later, it was found that especial precautions had to be taken in their care and feeding because of unaccountable variations in the water content of the tissues. Each dog, therefore, was kept in a separate kennel and given food and water by the same attendant and at regular intervals. Care also was taken to avoid large changes in the temperature of the room. No animals were used until kept for one month.

*Administration of the Anesthetic.*—Blood drawn from dogs under ether anesthesia fluctuated so greatly in its water relations that the results could not be used. Sodium barbital was therefore used in all the experiments reported. In some of the early work, barbital was injected intravenously, but fluctuations in water content of the blood persisted. However, when the anesthetic was given by stomach tube three or four hours before the experiment, no appreciable variations in water content occurred. Approximately 0.15 Gm. per kilogram of weight in 100 cc. of

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2. Briggs; D. R.: J. Phys. Chem. **35**:2914, 1931; also another article to be published in the same journal in January, 1932.

3. Rubner, M.: Abhandl. d. k. preuss. Akad. d. Wissensch., phys.-math. Kl., 1922, no. 1, p. 1.

4. Robinson, William: J. Biol. Chem. **92**:699, 1931.

water was used. At the time of the experiments, the animals were sleeping heavily and could be manipulated without being disturbed. Chart 1 shows the constancy of the water relations of the blood under these conditions during one and one-half hours. The symbols used on the charts are explained on page 5.

*Control Determinations.*—Control determinations were made for each dog used. Both arterial and venous blood was withdrawn under aseptic conditions from the dog one week before the experiment and under conditions closely similar to those of the experiment. Samples of blood were taken approximately every fifteen or

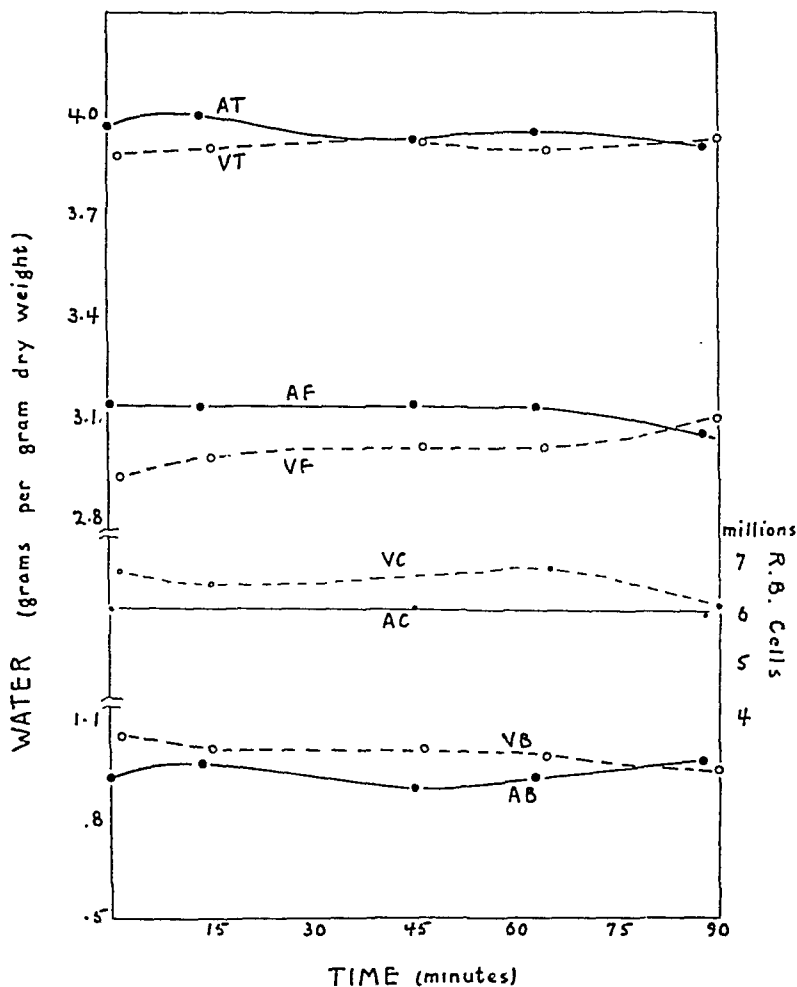


Fig. 1.—Typical control determinations of total (T), free (F) and bound water (B) of arterial (A) and venous (V) blood, with red cell counts (C). For further explanation of charts 1 to 9, see text page 5.

twenty minutes for about one and one-half hours. The dog was then given an intravenous injection of 500 cc. of 10 per cent dextrose to hasten recovery from the anesthetic. The effect of intravenous injections of dextrose on barbital-anesthetized dogs had been noted by Johnson, Lighthill and Luckhardt,<sup>5</sup> who found that they could not keep dogs asleep under barbital while dextrose was being administered.

5. Johnson, C. A.; Lighthill, J. A., and Luckhardt, A. B.: J. A. M. A. **95**: 576, 1930.

The blood thus drawn showed almost uniform values throughout the period. Chart 1 is typical of these control determinations that were made with each experiment.

The following week, when the dog was to be submitted to the crucial test, it was again placed under conditions closely similar to those of the experiment, and blood was again withdrawn at least twice for a second and partial control. Generally it was found that the water relations had not altered appreciably during the interval of the week. Again chart 1 is typical of all the control experiments.

*Experimental Technic.*—In all the experiments reported, blood was withdrawn from the left femoral artery and from the right femoral vein. These were exposed in order that the blood might be taken quickly. A cannula in the right carotid was attached to a manometer to record blood pressure. Blood was withdrawn in a syringe and at once transferred to petrolatum-coated tinfoil containers, which were then placed in glass vials and the vials corked, weighed and frozen.

When muscle was used, it was taken from a foreleg, and the exposed muscles were kept covered with hot, moist packs. For each test an entire muscle was dissected; thus the blood supply to the other muscles was least disturbed and the minimum amount of blood lost during removal.

For hemorrhage, blood was withdrawn in most instances from the left femoral artery in amounts varying from 100 to 200 cc. depending on the size of the dog.

For shock due to trauma, the left hind leg of the anesthetized animal was traumatized by blows from a 2-pound (0.9 Kg.) padded hammer, struck hard enough to bruise the soft parts without breaking bones or skin. The method is described by Parsons and Phemister.<sup>6</sup> When the left hind leg was traumatized, the arterial blood was collected from the left carotid artery; and as mentioned above the muscle was taken from a foreleg.

For the effects of histamine, doses of 2 cc. of 0.05 per cent histamine were injected into the left jugular vein.

#### THE CHARTS AND THE TABLE

*Charts.*—Triplicate determinations were made for every value shown on the charts. However, only the average of these is used, because of the large number of values that appear, and because deviations from the average were usually small. Deviations from the average do not necessarily indicate inaccuracies in measurement, since it should not be assumed that similar specimens taken from a tissue will all have the same total and bound water content. The extent of deviations from the mean was determined with a 10 per cent solution of gelatin in water. This was allowed to cool, small cubes were cut from the "gel" and determinations made in the usual manner. The average total water content was 8.344 Gm. per gram of dry gelatin, with a deviation of  $\pm 0.001$  Gm.; the bound water was 1.210 Gm., with a deviation of  $\pm 0.006$  Gm. It is therefore possible to obtain a fair degree of accuracy in this method.

In the charts the values for total, free and bound water are shown in grams per gram of dry weight. To find the value of water as a percentage of total weight, the number of grams of water is divided by itself plus 1 Gm. for dry weight and multiplied by 100. Thus, to convert the value 3.24 Gm. of water per gram of dry weight to a percentage basis:

$$\frac{3.24}{4.24} \times 100 = 76.4$$

6. Parsons, E., and Phemister, D. B.: Surg., Gynec. & Obst. **51**:196, 1930.

Percentage values for free and bound water are calculated on the basis of total water, and are obtained by dividing any value shown by the number of grams of total water and multiplying by 100.

The indications of the symbols used on the charts are as follows:

- B P*, blood pressure
- A T*, total water of arterial blood
- V T*, total water of venous blood
- M T*, total water of muscle
- A F*, free water of arterial blood
- V F*, free water of venous blood
- M F*, free water of muscle
- A B*, bound water of arterial blood
- V B*, bound water of venous blood
- M B*, bound water of muscle
- A C*, red blood cells of arterial blood
- V C*, red blood cells of venous blood

The values for arterial blood are shown on the solid lines, those for venous blood on the broken lines and those for muscle on the dotted lines.

*Table.*—The values given in the table are shown in double columns, as "grams per gram of dry weight" and as "percentages." In the percentage columns, the total water is calculated on the basis of total weight, and the free and bound water values on the basis of total water.

#### VARIATIONS IN WATER RELATIONS OF THE BLOOD WITH CHANGES IN CONCENTRATIONS OF RED BLOOD CELLS

A count of red blood cells was usually made each time blood was drawn. In the control period, the numbers remained fairly constant, but at the time of the crucial test they were frequently found to vary considerably. To determine what effect such fluctuations would have on the water relations of the blood the following experiment was conducted and repeated.

Freshly drawn heparinized blood was centrifugated; the plasma and corpuscles were separated, and mixtures of various concentrations of plasma and corpuscles were made as shown along the bottom of chart 2. Blood cell counts were made on mixtures containing up to 8,000,000 cells; the correlation between count and concentration is indicated by the oblique line.

The total water content of the plasma was found to be 91.6 per cent while that of the corpuscles was 66.3 per cent. In mixtures of two systems having such a wide difference in water content it would be expected that total water would be affected by changing the concentrations of the corpuscles; and this relation is shown in chart 2 by the solid line marked "total water." Variations in free water were closely similar to those of total, as appears from the broken line. The percentage of bound water in plasma was 17.6 per cent and that in the corpuscles 28.9 per cent; it will be seen that varying concentrations of corpuscles had little effect on the amount of water bound.



The two upright lines  $P_1$  and  $P_2$  mark off a section in which lie concentrations of red blood cells with counts from 3,500,000 to 8,000,000. Within these concentrations, the total water ranged from 86 to 76.4 per cent with a similar change in free water, while for bound water the change in values was almost negligible.

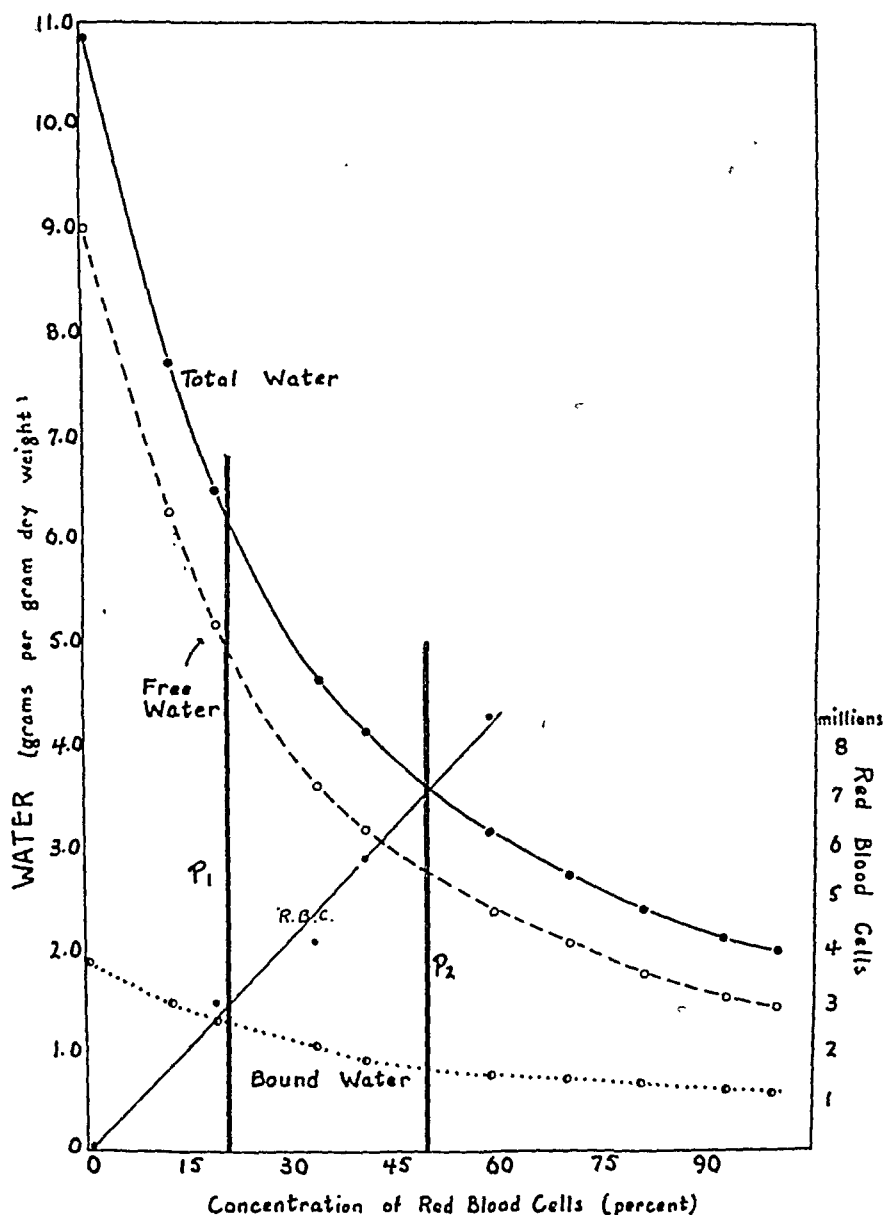


Fig. 2.—Values for total, free and bound water at various concentrations of red blood cells. The oblique line represents the correlation between the count and the concentration of red blood cells.

#### ALTERNATING WATER RELATIONS OF BLOOD AND MUSCLE

When specimens of muscle as well as of blood were taken, it was usually found that when a change in the total water of the blood was effected, the total water of the muscle was made to vary in the opposite

direction. These alternating values of the two tissues were found to concern largely changes in free water.

This effect is shown in charts 3, 4 and 5. It will be noted in chart 3 that when an increase in the percentage of total water of the blood

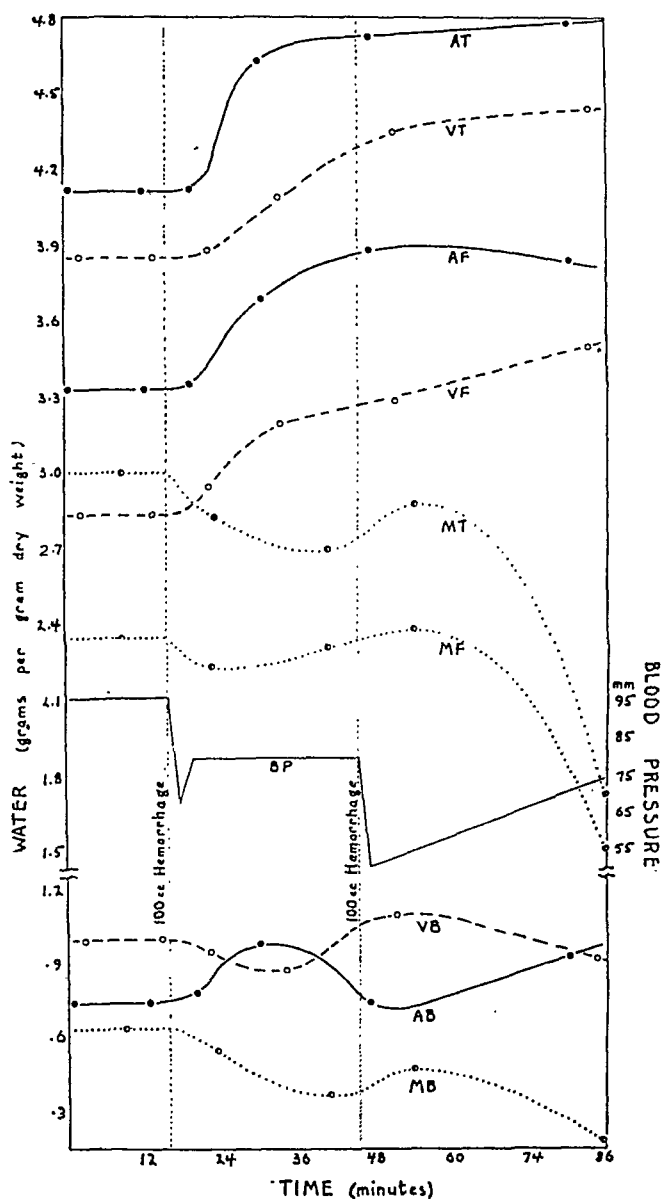


Fig. 3.—Effect of hemorrhage on the water relations of blood and muscle.

occurred, as after hemorrhage, a corresponding decrease took place in the water content of the muscle. The reverse movement is shown in chart 5; this occurred during "spontaneous" shock, when water left the blood and at the same time water entered the muscle. A similar movement followed the injection of histamine, but space does not permit the demonstration of this change in the chart.

The effect of a change in the total water of one tissue acted quickly to produce the reverse movement in the other tissue.

#### FLUCTUATIONS IN THE WATER CONTENT OF BLOOD AND MUSCLE, POSSIBLY DUE TO MUSCULAR ACTIVITY

In our early series, when the dogs were anesthetized on the experimental table, a considerable amount of intermittent struggling occurred

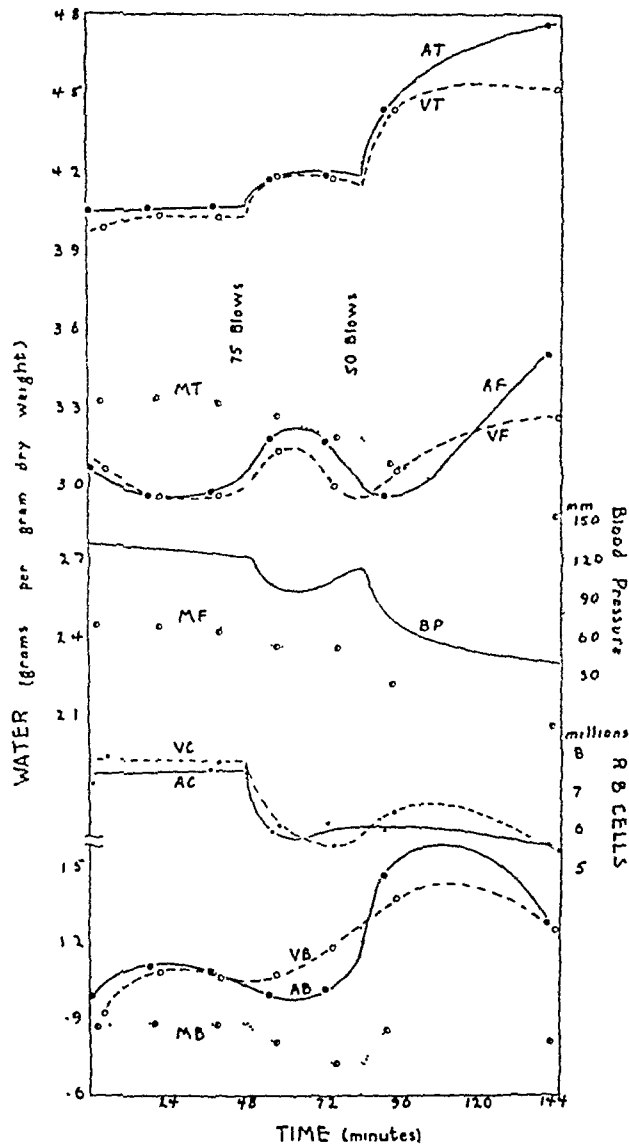


Fig. 4.—Effect of trauma to the leg on the water relations of blood and muscle.

during the experiment. Regularly, in such cases, it was noted that frequent fluctuations in the values of total, free and bound water occurred in the blood and muscles. A typical case is illustrated in chart 6, where a change of 1.2 per cent is shown to occur in the total water of the arterial blood and of 4.9 per cent in that of the venous blood. Bound water varied as much as 7.2 and 13.5 per cent, respectively.

In general, no alternation was noted here between the water content of the dissected muscle and that of the samples of the circulating blood taken at that time. It is possible that localized alternation occurs, but in our experiments this effect was probably obscured by the irregular and generalized activity.

No struggling took place, however, later, in the series, when the animals were given the anesthetic from two to three hours before the experiment began. These dogs were sleeping heavily when placed on

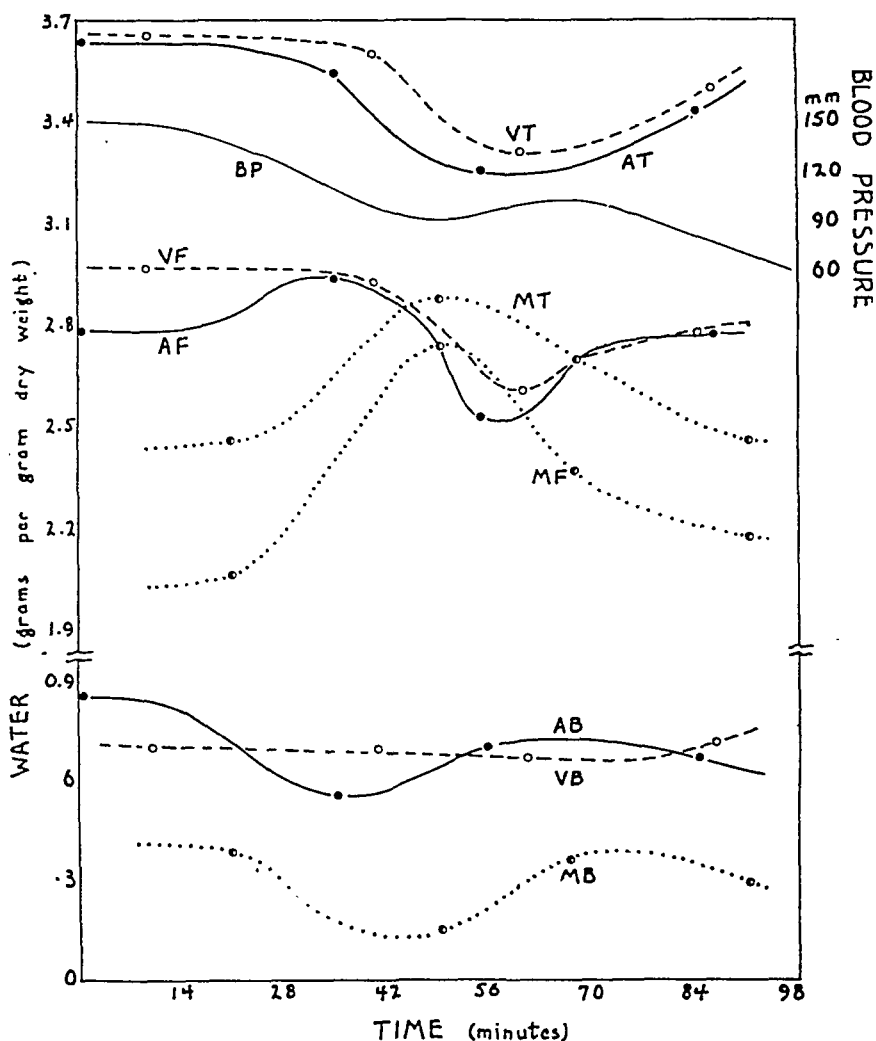


Fig. 5.—Changes in the water relations of blood and muscle during a spontaneous fall in blood pressure.

the table. In these later cases, moreover, no variations in the water content were observed; the almost uniform values obtained are shown in chart 1.

The assumption is therefore made that strenuous muscular activity may affect the water relations of the muscles and also of the blood.

## EFFECTS OF HEMORRHAGE

The effects of hemorrhage were observed on six controlled animals. Blood was withdrawn usually twice, several minutes apart, and in amounts ranging from 100 to 200 cc., according to the size of the dog. The percentage of total water of both arterial and venous blood increased regularly after the first, and sometimes following the second, withdrawal. This phenomenon is shown in charts 3 and 7.

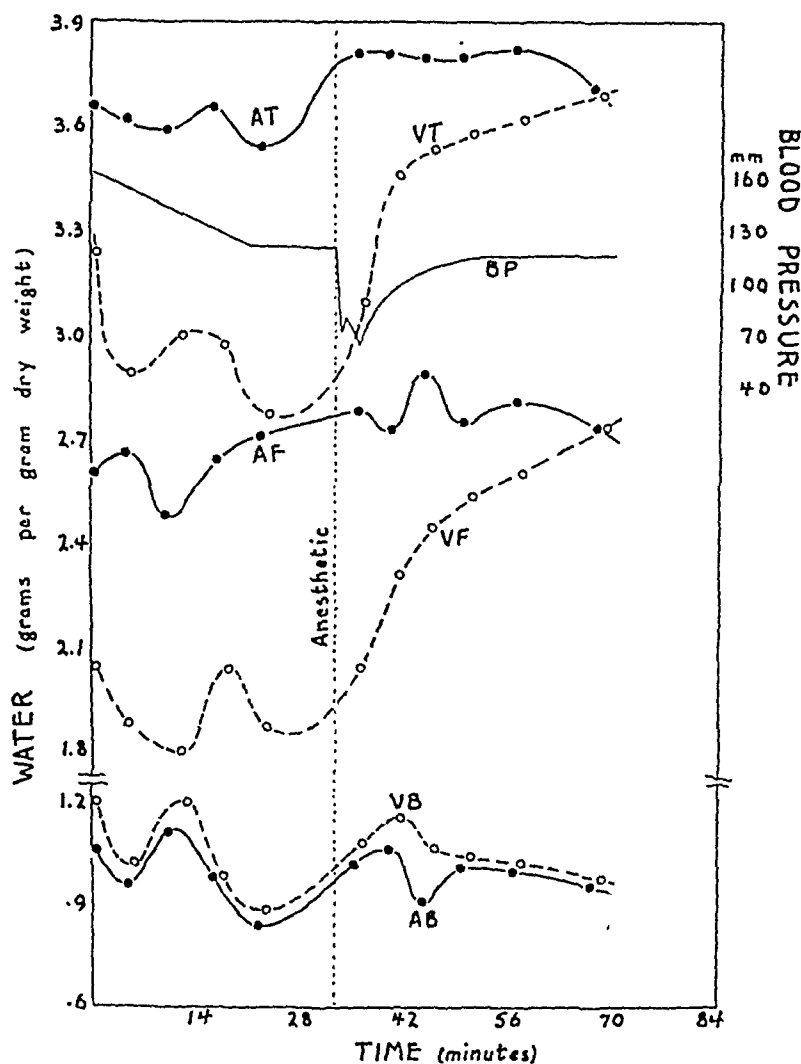


Fig. 6.—Fluctuations in the water relations of blood evidently the result of muscular activity.

In the experiment illustrated in chart 7, in which a dog weighing 11 Kg. was used, increases in total, free and bound water in the blood were shown to take place after each hemorrhage. The total water of both arterial and venous blood rose from 4.1 to 5 Gm. per gram of dry weight, which is an increase from 80.2 to 83.4 per cent. The free and bound water increased in smaller amounts, their sum in grams per gram of dry weight at any point being equal, of course, to the value shown for total water.

Specimens of muscle in addition to blood were taken in the experiment illustrated in chart 3, in which a dog weighing 12 Kg. was used. The percentage of total water is shown to increase in the blood following hemorrhage, but at different rates in the arterial and venous blood. Similar changes are seen for free water, while the bound water values for arterial and venous blood vary alternately.

With muscle, the effect of hemorrhage was evident in a general fall in total, free and bound water. The total water content fell from 3 to

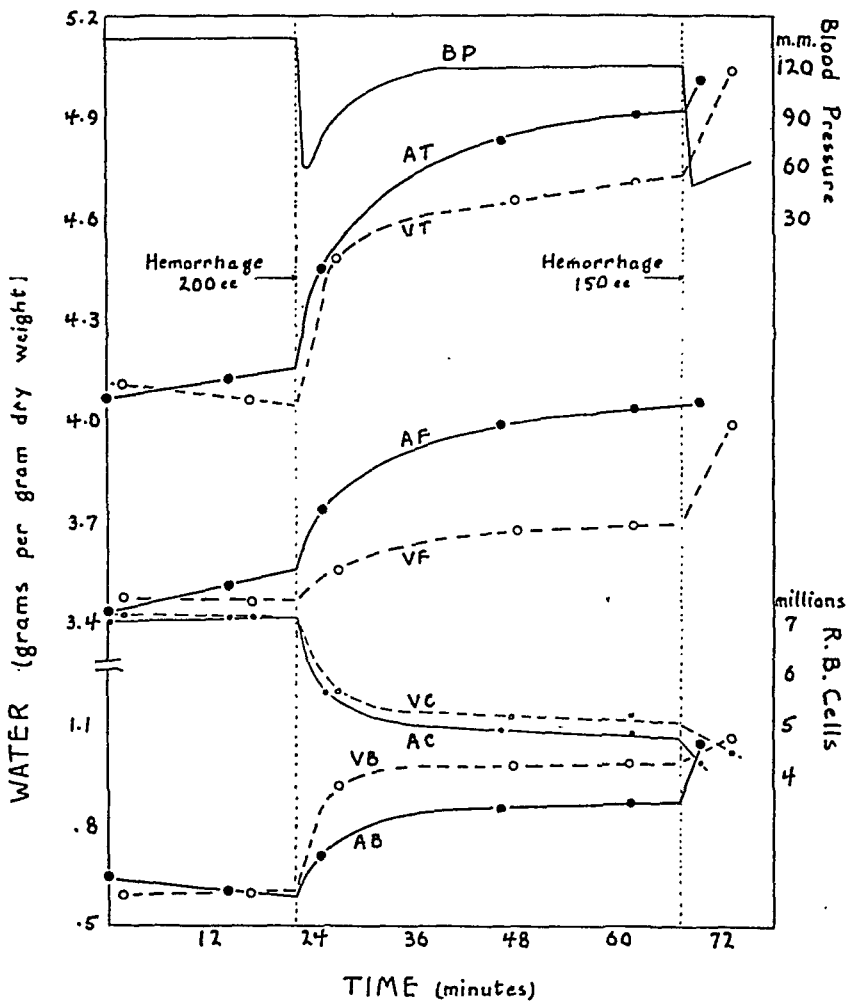


Fig. 7.—Effect of hemorrhage on the water relations of blood.

1.7 Gm. per gram of dry weight, or from 75 to 63.2 per cent, being a loss of 11.8 per cent. This is a commonly observed phenomenon, namely, that hemorrhage causes a desiccation of the tissues other than blood, the water of the tissues, especially of the skeletal muscles, passing into the blood to replace its lost volume. The passage of water from the skeletal muscles to the blood was, in this instance, largely a transfer of free water; but at the same time, the bound water fell in a similar manner, though in a smaller proportion.

Blood pressure fell from 96 mm. of mercury to 67 and 50 mm., respectively, after the first and second withdrawals, to regain a pressure of 74 mm. forty minutes later.

#### EFFECTS OF THE INJECTION OF HISTAMINE

These tests were made for comparison of the effects with those resulting from traumatism of a limb. In this series, four dogs were

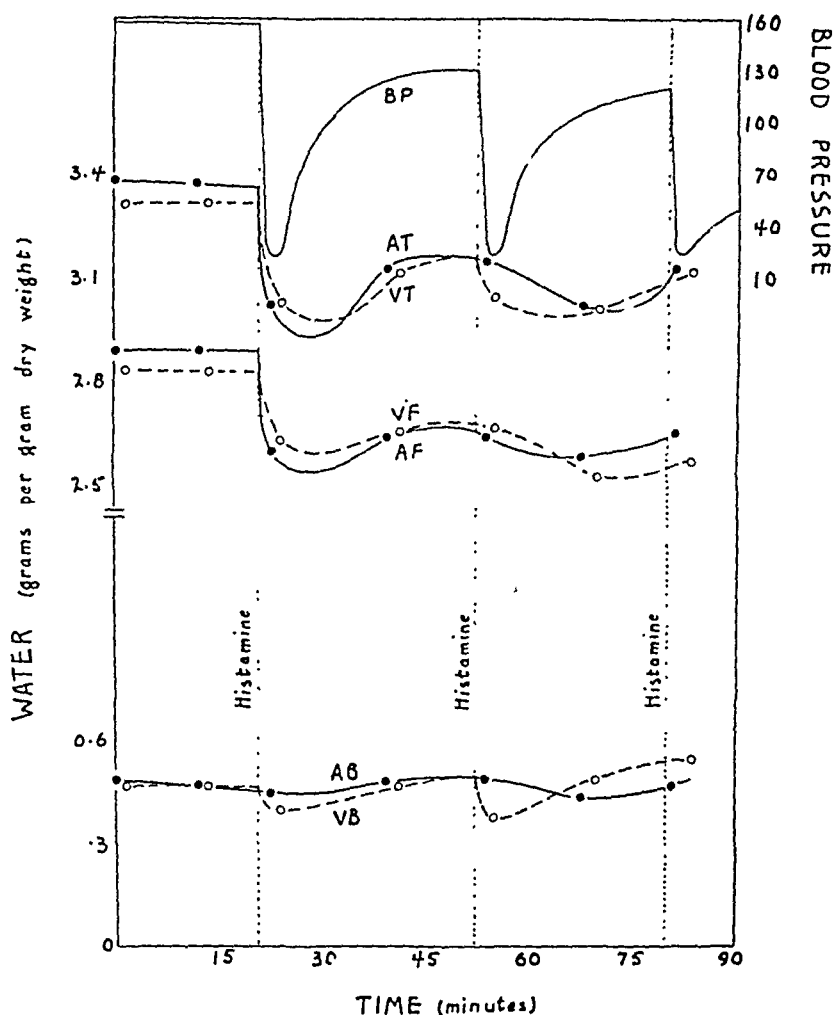


Fig. 8.—Effect of injection of histamine on the water relations of blood.

used. The changes typically found are shown in chart 8. Quickly following the first injection of histamine, the total water content of both arterial and venous blood fell from 3.3 to 3 Gm. per gram of dry weight, which was a decrease of about 2 per cent of water. A partial recovery took place, and at the time of the second injection another, but less marked, fall occurred. These fluctuations were largely changes in free water. Blood pressure fell in a characteristic manner after each injection and was followed by a slow and incomplete recovery, which always failed to reach the normal level.

The red blood counts typically rose from a normal value of about 5,760,000 to about 6,080,000 after the first injection, and increased after the second and third injections until a count as high as 8,400,000 had been reached. This, of course, is to be expected after the marked movement of water from the blood to the tissues that follows the injection of histamine.

In all cases in which specimens of muscle were taken for analysis of water content before and after injection of histamine, it was found that the muscle gained at about the same rate that the blood lost water.

#### EFFECTS OF TRAUMA

The six dogs used in this series may be separated into two groups according to the initial effect of trauma on the water content of the blood. The effect in the first group, which consisted of four dogs, is represented by chart 9. This was an experiment on a normal dog weighing 14 Kg. After the first series of blows, the total water content of both arterial and venous blood gradually fell from 3.5 to 3.2 Gm. per gram of dry weight, which was a loss of 1.5 per cent of total weight. However, following the second trauma, the outgo of water from the blood ceased, and a rapid rise began. During the next thirty minutes, in which a third series of blows was given, the water rose to approximately 4 Gm., an immediate increase of 3.5 per cent of total weight, and a net increase of 2 per cent over the initial value.

This movement of water was usually of free water, the bound water remaining almost constant, although in one animal the bound water rose after the second trauma from 0.35 to 1 Gm. per gram of dry weight, an increase of 9.3 per cent of total water. The blood pressure fell slightly following the first trauma and while the water was leaving the blood.

In the experiment illustrated in chart 4, the blood pressure fell about 20 mm., but regained most of this before the second trauma. A much greater fall occurred after the second and third series of blows, when the pressure dropped from about 144 to 30 mm. of mercury in about thirty minutes. Counts of red blood cells were not obtained in all cases. When taken, they indicated a slight concentration at the time of the first trauma coincident with the loss of water and fall in blood pressure. Counts also showed a dilution when the blood regained water, as is seen under conditions of hemorrhage.

During the period of trauma, which lasted for from one to three hours, the traumatized leg always became markedly discolored and swollen. This phenomenon was also noted by Parsons and Phemister<sup>6</sup> in a large series of experiments on trauma to the leg, and they observed that the swelling was accompanied by a progressive fall in blood pressure. They weighed both the traumatized and the normal leg and found that



the injured one was much the heavier. On dissection, they found the tissues of the traumatized leg to be edematous and engorged with blood.

Further effects of severe trauma were shown in the second group, which consisted of two dogs; here a rise instead of a fall occurred in the total water of the blood following the first series of blows. This is shown in chart 4, the animal used weighing 21.4 Kg. The increases of 2.5 per cent that took place in the total water of the arterial blood and

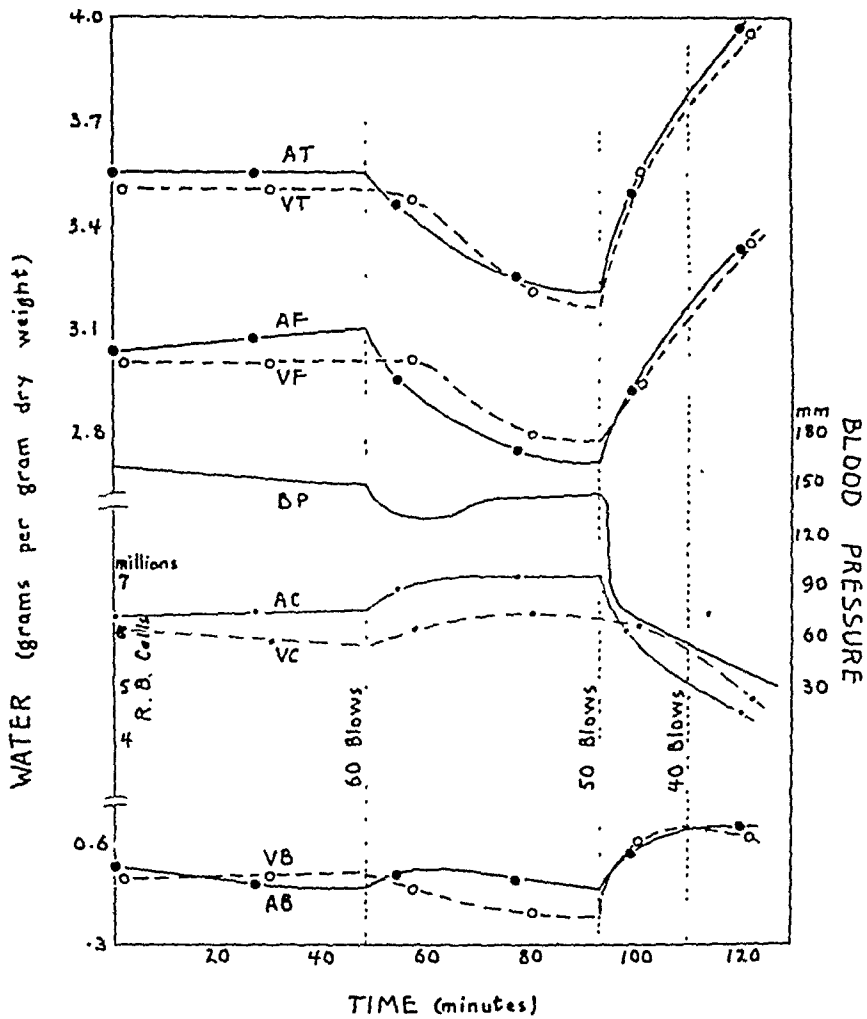


Fig. 9.—Effect of trauma to the leg on the water relations of the blood.

of 2 per cent in that of the venous blood at the termination of the test were similar to those seen in the first group of dogs. Bound water increased 8.7 per cent in the arterial and 4.4 per cent in the venous blood after the second series of blows, but fell somewhat at the end.

Specimens of muscle were taken in this experiment, and as usual were cut from the left foreleg, the trauma being administered to the right hind leg. The alternating values of the water contents of the blood and muscles noted in a previous section as a general phenomenon are seen also in this chart, which shows that as the proportion of water

of the blood rose that of the water of the muscles fell. The change was largely in the free water content.

Blood pressure fell slightly after the first trauma, recovered almost completely and fell again following the second series of blows from nearly 120 to about 40 mm. of mercury. The number of red blood cells decreased in the manner usually noted following hemorrhage.

#### OTHER "SHOCKLIKE" CONDITIONS

A case of "spontaneous" fall in blood pressure is included to show the changes in the water relations that ensued. The experiment is

#### *Maximum Changes That Occur in the Water Relations of Blood and Muscle Following Hemorrhage, Injection of Histamine and Trauma to the Leg*

	Total Water		Free Water		Bound Water		Comment
	Gm. per Gm. Dry Weight	Percent- age of Total Weight	Gm. per Gm. Dry Weight	Percent- age of Total Water	Gm. per Gm. Dry Weight	Percent- age of Total Water	
Normal arterial blood	4.11	80.4	3.37	82.0	0.74	18.0	See chart 3
1 hr. after hemorrhage	4.78	82.7	3.99	84.4	0.92	19.2	
Normal venous blood	3.86	79.4	2.87	74.4	0.99	25.6	
1 hr. after hemorrhage	4.44	81.6	3.53	79.5	0.91	20.5	
Normal muscle from foreleg	3.00	75.0	2.37	79.0	0.63	21.0	See chart 3
1 hr. after hemorrhage	1.77	63.2	1.54	89.5	0.18	10.5	
Normal arterial blood after injection of histamine	3.38	77.2	2.89	85.5	0.49	14.5	See chart 8
	3.01	75.1	2.57	85.4	0.44	14.6	
Normal venous blood after injection of histamine	3.32	76.8	2.85	85.9	0.47	14.1	
	3.01	75.1	2.52	83.7	0.49	16.3	
Normal arterial blood	4.05	80.2	3.06	75.6	0.99	24.4	See chart 4
1½ hr. after trauma	4.77	82.7	3.49	73.2	1.28	26.8	
Normal venous blood	4.04	80.1	2.95	73.0	1.09	27.0	
1½ hr. after trauma	4.53	81.9	3.26	72.1	1.26	27.9	
Normal muscle	2.45	71.0	1.58	64.5	0.87	35.5	See chart 4
1½ hr. after trauma	2.07	67.4	1.26	60.9	0.81	39.1	

represented in chart 5. When the animal had been anesthetized, and specimens of blood and muscle were being taken, a steady fall in blood pressure, from 150 mm. to 120 mm. of mercury was noted. The decrease continued for forty-five minutes; at that time it had reached 92 mm. A slight recovery occurred, but terminated in a fall to 60 mm. During the first fall in blood pressure, the total water of both arterial and venous blood decreased from 78.4 to 76.8 per cent, while the water of the muscle increased from 71.1 to 74.2 per cent, indicating that a loss of liquid from the blood to the fixed tissues was involved in the fall of blood pressure. In about fifty-five minutes, the blood began to gain and the muscles to lose water, associated with which occurred a temporary rise in blood pressure. Some fluctuations in the bound water occurred during the period, but the changes were mostly in the free water.

The cause of the circulatory failure in this animal is not obvious. It occurred in one of the earlier experiments in which the sodium barbital was injected intravenously, a preliminary dose of morphine having been administered hypodermically. In this dog, the muscle for analysis was removed from the abdominal wall instead of from the foreleg, and the peritoneum was injured, in one place being torn so that the intestines extruded.

While the question of circulatory failure resulting from abdominal operation is not within the scope of this report, the changes shown in this experiment were similar to those noted in so-called surgical shock, and showed that when the water left the blood, it entered the muscle.

#### COMMENT

In these experiments, the circulatory embarrassment or failure that occurs after hemorrhage, trauma to an extremity or injection of histamine was followed by quantitative determinations of the total, free and bound water in the circulating blood and skeletal muscles. This method of studying water relations is reported as an adjunct to the investigation of the physiologic and pathologic changes involving blood volume, edema and dehydration.

Previous to this study, the method had not been employed over a period of time, with changing conditions in the blood or other tissues; consequently, no other results are available for comparison. Values for total water content alone evidently do not give an adequate picture of the changes that occur. Such data, however, may have more significance when accompanied by determinations of the degree with which the water is bound by the colloids and dissolved substances present.

In the control tests, when blood was withdrawn from the dogs, without disturbing them, during a period of from one and a half to two hours, it was found that the total, free and bound water of the blood and muscles remained practically constant throughout the period. In addition, similar values were found one week later, when the animals were again tested. This uniformity was observed over a period of a week in numerous cases, and leads to the conclusion that it is a normal condition. Any changes in water content, therefore, such as those that are demonstrated to follow hemorrhage, trauma and injection of histamine, appear to have some significance, although the observed changes are usually not more than from 3 to 5 per cent.

Changes in the water content of blood or muscle, which may appear to be small when calculated on a percentage basis, may nevertheless represent a proportionately large volume of water. For instance, in a 10 Kg. dog, the muscle of which, containing 75 per cent water, makes up 45 per cent of the total weight of the dog, a change of 5 per cent in

the water content of the muscle would involve a movement of 225 cc. of water. In the case of the blood, containing 80 per cent water and making up about 7.7 per cent of the total weight of the dog, a change of 5 per cent in the water content would mean a change in volume of 38.5 cc.

The relative proportions of water in the muscle and in the blood are approximately as 225 is to 38. Therefore if, say, 5.9 per cent of the water of the blood should move to the muscle, it would raise the water content of the muscle by only 1 per cent.

A comparison of values for free and bound water in tissues obtained by different investigators seems to be of limited worth, because the values are derived by methods that have not been standardized. The various methods employed at present in the study of bound water have recently been discussed by Briggs.<sup>7</sup> However, it is noted here that Hill,<sup>8</sup> using a vapor pressure method, found about 97 per cent of the total water of the blood to be free water, in that it could act as a solvent for salts; he found the same percentage of free water in the muscles. In the determinations reported here, the amount of free or freezable water at  $-20^{\circ}\text{C}$ . varies in the control animals from 65 to 85 per cent.

Compensation for a nonfatal hemorrhage soon sets in; the plasma in the blood is renewed, and the blood regains its normal volume within a few hours in slight hemorrhage. The red blood corpuscles and the hemoglobin, however, are restored more slowly, requiring a number of days or several weeks. The rapidity with which the plasma is restored is shown by the increase in the total water content of the blood. The increase is largely in freezable water, but there is also an appreciable rise in bound water. The water, in large part, comes from the muscles, as shown by a decrease in the water present there. The rate at which the interchange of fluids takes place indicates that the water is loosely bound in a physical sense, and that the walls of the blood vessels are easily permeable.

The action of histamine appears to be on the capillary wall, which Krogh<sup>9</sup> claimed to be actively contractile and to have an intrinsic tone that can be modified by nervous and chemical influences. The dilation of the capillaries produces a stasis in the capillary bed and permits extravasation of the plasma. The circulating blood shows a concentration of corpuscles and a decrease in water content, the low blood volume producing the fall in blood pressure. Dale,<sup>10</sup> one of the discoverers and first investigators of histamine, pointed out the resemblance of certain states of traumatic shock to the effects of injection of histamine.

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7. Briggs (footnote 2).

8. Hill, A. V.: *Proc. Roy. Soc. London, s.B.* **106**:477, 1930.

9. Krogh, A.: *The Anatomy and Physiology of Capillaries*, New Haven, Conn., Yale University Press, 1924.

10. Dale, H. H.: *Bull. Johns Hopkins Hosp.* **30**:257, 1920.

"Traumatic" shock (so-called because it is a secondary shock that usually develops slowly following an injury with little or no obvious hemorrhage) has been the subject of much investigation. The studies of the Medical Research Committee during the Great War were summarized in a monograph by Cannon.<sup>11</sup> The experimental method that produced a low blood pressure and other phenomena most nearly similar to the condition seen in the injured soldier consisted in traumatizing one of the posterior extremities of an animal. The theory that the committee thought satisfied the findings was that of traumatic toxemia. This presumes the formation of a toxic substance in the bruised or injured tissue, which enters the blood stream to produce a capillary dilatation and the resultant lowering of blood pressure. Since histamine is a toxic substance that acts in this manner when injected into the blood, the toxic agent was thought to be histamine-like.

Recently the theory of traumatic toxemia has been questioned, and further investigations have failed to prove the presence of a toxic agent either in the circulating blood or in the traumatized tissue of an animal the extremity of which has been injured. Parsons and Phemister<sup>6</sup> repeated the experiments of Bayliss and Cannon on a large number of dogs and found that traumatism of the limb is accompanied by a general anemia and a corresponding increase in the limb volume, which is largely due to hemorrhage into the damaged tissues. They found no evidence of a toxic, blood pressure-lowering substance, and considered it preferable to speak of hemorrhage or of shock due to hemorrhage, rather than to use the term "shock" when acute loss of blood in open or closed wounds is the cause of marked circulatory failure or embarrassment.

Blalock,<sup>12</sup> using the same experimental method, found that blood pressure could not be reduced to the shock level by trauma to a posterior extremity without causing the loss of a sufficient part of the blood volume into the traumatized area to account for the decline in pressure. He found a greater proportionate loss in plasma than in red cells, which accounted for the concentration of the blood elsewhere. The important demonstration by Keith and others<sup>13</sup> of the reduction in the amount of the circulating blood in secondary shock has not been sufficiently emphasized in explaining the condition, although it has been used as a basis for the treatment of persons suffering from secondary shock.

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11. Cannon, W. B.: *Traumatic Shock*, New York, D. Appleton and Company, 1923.

12. Blalock, Alfred: *Arch. Surg.* **20**:959, 1930.

13. Keith, N. B.: Medical Research Committee, Reports of the Special Investigation Committee on Surgical Shock and Allied Conditions: IX. Blood Volume Changes in Wound Shock and Primary Hemorrhage, London, His Majesty's Stationery Office, 1919, Special Report Series, no. 27.

In comparing the changes in the amount of water in the blood following trauma to the leg with the changes after injection of histamine, it is seen that in a number of animals there is a similarity, in that an initial decrease takes place in the water in the circulating blood. This is explained in the case of the injection of histamine on the basis of capillary dilatation with increased capillary permeability.

In the case of injury to the leg there is a localized erythema with capillary dilatation immediately following the hammering of the leg. It is assumed that a loss in plasma takes place before the extravasation of erythrocytes occurs. Actual hemorrhage into the swollen tissues of the leg is a slower process. In our experiments, the effect was hastened by a second trauma, after which occurred a marked swelling of the leg and a fall in blood pressure. The circulating blood became diluted, the muscles lost water, and the result was a more watery blood—a condition that exists after hemorrhage. In the animals in which the injury to the leg was followed immediately by an increase in the water content of the circulating blood, the blows of the hammer were probably sufficient actually to rupture vessels, so that bleeding occurred rapidly into the tissues of the leg, producing the characteristic water changes after hemorrhage.

#### CONCLUSIONS

A study has been made of the water content of the blood and muscles in respect both to total water and to water in the "free" and in the "bound" state, in animals under the influence of hemorrhage, histamine and trauma. The observations show that with hemorrhage there is a compensatory transfer of water chiefly in the "free" state from the muscles to the blood. In "histamine shock," the fall of blood pressure is associated with, and perhaps is partly the result of, a transfer of water from the blood to the muscles. In "shock" produced by severe traumatism to a leg, the evidence indicates that with minor trauma there is in some cases a loss of fluid from the blood to the muscles associated with a fall in blood pressure, and that with major trauma the picture is that of hemorrhage, in that there is a dilution of the blood remaining in the general circulation with water withdrawn from the muscle. These observations are in support of the work of Parsons and Phemister and others which indicates the importance of the loss of blood in traumatized tissue in causing the fall of blood pressure noted after severe traumatism.

#### SUMMARY

In hemorrhage, injection of histamine and shock due to hemorrhage, an alternation exists between the water content of the blood and that of the skeletal muscles. That is, under these conditions, when an increase occurs in the water of the blood, a decrease takes place in the

water of the muscles, and vice versa. The changes are almost simultaneous, and in some cases seem to be proportionate.

Generalized muscular activity produces slight but frequent fluctuations in the water content of both blood and muscles, sometimes increasing and at other times decreasing the percentage of water present. The alternation noted in the preceding paragraph was not observed under these conditions.

A hemorrhage of from 200 to 300 cc. of blood in dogs weighing from 10 to 14 Kg. produces an increase in the percentages of the total and free water contents of the blood. That this indicates a dilution of the blood is shown by the decrease in the erythrocyte count. A fall in the total, free and bound water in the muscles accompanies the rise in the water of the blood.

The sudden fall in blood pressure that follows the injection of histamine is accompanied by a decrease in the total and free water of the blood and small changes in the bound water. The circulating blood becomes concentrated, as shown by both the decrease in water content and the increase in the erythrocyte count. With the loss of water from the blood occurs a rise in the water of the muscle.

In trauma to the leg, in some dogs, the water in the blood is decreased after the first trauma, owing to passage of water from the blood to the general skeletal muscles, and is increased following a second trauma, probably because of the rupture of blood vessels and consequent internal hemorrhage. In other dogs, no preliminary concentration of the blood occurs, but a dilution as is seen in hemorrhage. In all dogs, after the traumatized leg has become markedly swollen, there occur an increase in the water of the blood, a decrease in that of the muscles distant from the trauma, and a decrease in the number of circulating erythrocytes, indicating hemorrhage in the limb. The condition at the end of the experimental trauma shows water relations similar to those that follow hemorrhage.

# NONSYPHILITIC AORTIC VALVE DEFORMITY\*

B. J. CLAWSON, M.D.

MINNEAPOLIS

There are two kinds of nonsyphilitic diseased aortic valves: those with active vegetations and those that are deformed from thickening and roughening due to scar tissue and, in many cases, to calcium deposits.

The term valve defect was used by Libman<sup>1</sup> and others to denote a healed condition of the valve in contrast to active inflammation of the cusps as seen in acute rheumatic or bacterial endocarditis.

Healed aortic valve deformities are sometimes incorrectly diagnosed. Not infrequently the stigma of syphilis is unfairly attached to a person who has an aortic valve deformity, nonsyphilitic in origin. Both clinicians and pathologists make diagnoses of arteriosclerotic valve deformity. The concept of an arteriosclerotic aortic valve deformity depends, in general, on the work of Mönckeberg<sup>2</sup> who, after studying aortic valves in which there was extensive calcification, decided that the process bringing about the change in the cusps was the same as that found in the intima of the aorta in senile arteriosclerosis. Mönckeberg held the opinion that the arteriosclerotic process extended from the aorta to the valves by way of the sinus of Valsalva. His observations suggested that the change in the cusps was most pronounced at the angle where the cusps and the aorta join. Margolis, Ziellessen and Barnes<sup>3</sup> concluded that the etiology and pathogenesis of this type of valve lesion could not be determined with certainty, but that clinical and anatomic data indicated that in some cases the lesion may have an inflammatory basis, whereas in others it may be the result of a noninflammatory, degenerative process.

The frequency of cases coming to autopsy with a healed calcified nodular aortic valve lesion and the diversity of opinions among internists and pathologists concerning the frequency, etiology and pathogenesis of these lesions led to a study of healed aortic valve deformities

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\* From the Department of Pathology, University of Minnesota.

1. Libman, E.: The Clinical Significance and Course of Subacute Bacterial Endocarditis, *Brit. M. J.* **2**:30, 1920.

2. Mönckeberg, J. G.: Der normale histologische Bau und die Sclerose der Aortenklappen, *Virchows Arch. f. path. Anat.* **176**:472, 1904.

3. Margolis, H. M.; Ziellessen, F. O., and Barnes, A. R.: Calcareous Aortic Valvular Disease, *Am. Heart J.* **6**:349, 1931.



in autopsy material of the department of pathology at the University of Minnesota. In this study special emphasis was placed on the so-called arteriosclerotic valve as described by Mönckeberg.<sup>2</sup> This type of aortic valve deformity was called the calcified nodular type by Clawson and Bell in a former paper.<sup>4</sup>

An effort is made in this work to see if arteriosclerosis of the valve is ever of a degree severe enough to be responsible for cardiac failure, and to determine, if possible, the etiology and pathogenesis of calcified nodular aortic valve deformity. This type of lesion will be spoken of as the calcified nodular aortic valve deformity, to distinguish it from the syphilitic aortic valve deformity and from the healed non-calcified rheumatic lesions of the aortic valve.

The calcified nodular type of aortic valve deformity deserves consideration from two angles: (1) the frequency of the lesion with

TABLE 1.—*Degree of Stenosis in Sixty-Eight Cases of Calcified Nodular Aortic Valve Deformity*

Degree of Stenosis	Cases	
	Number	Per Cent
0	6	9.0
+	17	25.0
++	16	23.5
+++	29	42.5
Total.....	68	100

Grade + is the least stenosis which can be detected anatomically. Grade +++ is the most severe stenosis; with such a degree the aortic orifice is almost closed. Grade ++ is intermediate between grades + and +++.

aortic insufficiency or stenosis and (2) the etiology of the lesion. The former consideration is of much significance to the internists, and the latter chiefly concerns the pathologists.

#### FREQUENCY

Aortic insufficiency is generally admitted to be a not infrequent condition. Aortic stenosis has been looked on as a condition that seldom occurs. Osler<sup>5</sup> referred to it as rare. My findings are not in agreement with this idea.

In the autopsy material that I studied there were represented 253 deaths due to lesions of the cardiac valves, and in 161 (63.6 per cent) of the cases there was aortic valve deformity, either alone or in association with deformities in other valves. Ninety-three (57.7 per cent) of the cases in which there was aortic valve deformity were of the Mönckeberg

4. Clawson, B. J., and Bell, E. T.: 'Valvular Diseases of the Heart, Am. J. Path. 2:192, 1926.

5. Osler, W.: The Principles and Practice of Medicine, New York, D. Appleton and Company, 1925, p. 831.

type, or what I have called the calcified nodular type. In 90 per cent of the cases in which there was aortic valve deformity alone, it was of the calcified nodular type. In the cases in which the aortic valve deformity was severe, the calcified nodular type was the one generally found.

The frequency of a relatively marked grade of stenosis was high. Table 1 shows the degree of stenosis found in 68 cases examined from the 93 cases presenting calcified nodular valve deformities.

It is seen in these 68 cases of calcified nodular aortic valve deformity that some degree of stenosis is present in 62 (91 per cent). Stenosis of grades ++ and +++ can generally be detected by physical signs (based on the histories of the cases). It appears that about 66 per cent of the cases of calcified nodular aortic valve deformity show aortic stenosis clinically. It becomes evident from the foregoing figures that at least 38 per cent of all nonsyphilitic aortic valve deformities or 24 per cent of all nonsyphilitic old valve defects will show stenosis clinically. This frequency is much higher than is generally believed. The frequency of stenosis with aortic valve deformity may even be greater than 38 per cent, since it is possible to have an aortic stenosis with noncalcified aortic valve deformity. This, however, in my experience, is not common, since a nonsyphilitic aortic valve deformity of marked severity usually becomes calcified. It should be borne in mind that aortic stenosis is common. This is of significance to the internist in making a diagnosis of the type of valve injury in a case in which death is due to valve deformity. Regurgitation is commonly associated with the stenosis, but stenosis occurring alone is frequent.

#### ETIOLOGY

There has been much discussion concerning the origin and pathogenesis of the calcified nodular aortic valve deformities. Some observers believe that the thickened, calcified aortic cusps are the end-result of repeated attacks of rheumatic valvulitis with scar formation and calcification similar to that in inflammatory scar tissue in other valves and in other parts of the body. Others look on the aortic lesions as a pure metabolic process. Mönckeberg<sup>2</sup> considered the changes in the valves a part of the phenomenon of arteriosclerosis. Libman also described the lesions as arteriosclerotic in origin. The lesions in the 93 cases concerned here are studied from both the infectious and the metabolic angles to see which of the two factors seems more likely to be the etiologic agent. In attempting to arrive at the probable etiologic basis, important data can be collected from the histories of the cases, such as age and a history of acute rheumatic fever, and from the autopsies, such data as evidence of pericarditis, the condition of the arch of the aorta, frequency of defects of other valves and the gross and microscopic structures of the valves themselves.

Those who believe that these calcified nodular valve deformities are due to faulty metabolism, which results in calcification of the thickened cusps independent of an associated inflammation, stress the fact that this condition in the aortic valve is most common in older people. It is therefore assumed that these lesions are part of a senile process.

In order to study the relation of calcified nodular aortic valve deformities to age, a comparison of the age incidence of the calcified nodular aortic deformity is made with the age incidence of valve deformities that did not present the calcified nodules in the aortic cusps. By referring to figure 1 it is seen that death occurred in the group

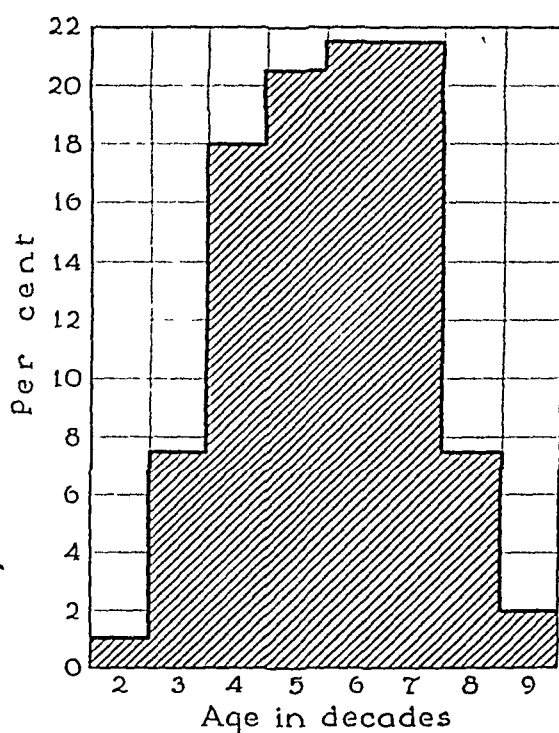


Fig. 1.—Age incidence of calcified nodular aortic valve deformity (93 cases).

with the calcified nodular aortic valve deformities most frequently in the sixth and seventh decades (43 per cent). In only 26 per cent of the group did death occur in the second, third and fourth decades, while in 74 per cent it occurred in decades 5 to 9. Yet it is to be observed that 47 per cent of the persons in this group died by the time they were 50 years old. The disease, obviously, cannot be considered entirely a disease of old age.

Figure 2 shows the ages at which death occurred most frequently in 160 persons not having calcified nodular aortic valve deformities. The greatest number of deaths occurred in the fifth decade, or about 15 years earlier than in the group with calcified nodular aortic valve deformities. Forty-four per cent of the group without the calcified

aortic valves died in decades 1 to 4, while 56 per cent died in decades 5 to 8. It is evident that death occurs at an older age in the group with calcified nodular aortic valve deformities than in the group in which such aortic deformities are not present. The question arises whether senile disease is the deciding factor in the pathogenesis of the valve deformities in this group or whether persons in this group live

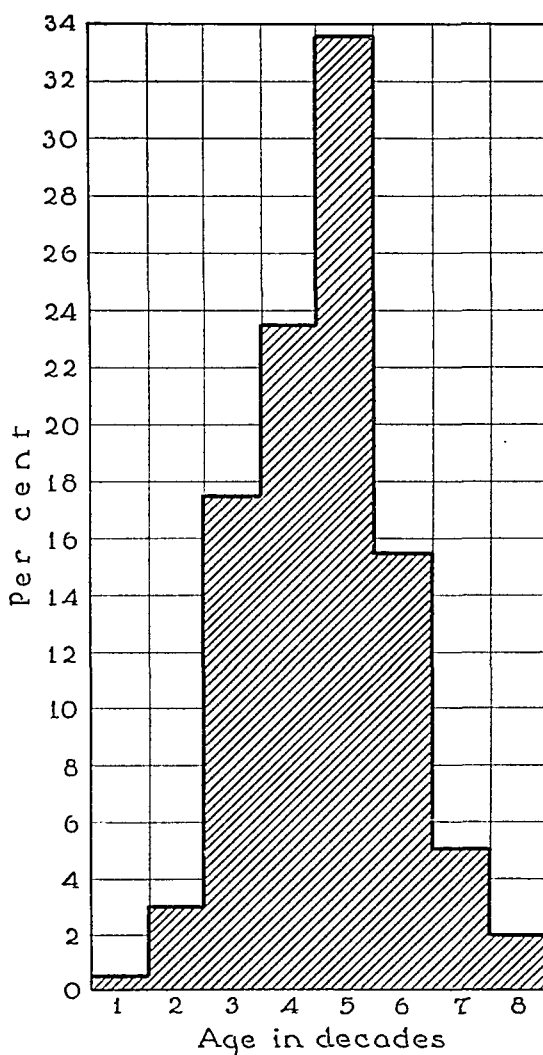


Fig. 2.—Age incidence of old rheumatic valve deformity (160 cases).

longer because the aortic valve is chiefly involved and because the left ventricle can stand an aortic lesion better than a mitral lesion. This question is apparently partly answered by referring to figure 3, which is based on 34 of the 93 persons with the calcified nodular aortic valve deformity. These 34, besides having the aortic lesion, also had a mitral valve deformity of greater or less degree. The greatest number of these 34 died in the fifth decade instead of in the sixth and seventh decades, as did the greatest number of the 93 having the calcified

nodular aortic valves. The percentage of those dying before the fifth decade was 35 instead of 26, and the percentage of those who died in decades above the fourth was 65 as compared with 74. The important thing to be observed in comparing figures 1 and 3 is that with mitral involvement death takes place at an earlier period than when the aortic valve alone is involved. It is also to be noted in observing figure 1 that these calcified nodular aortic valves can be present in relatively young

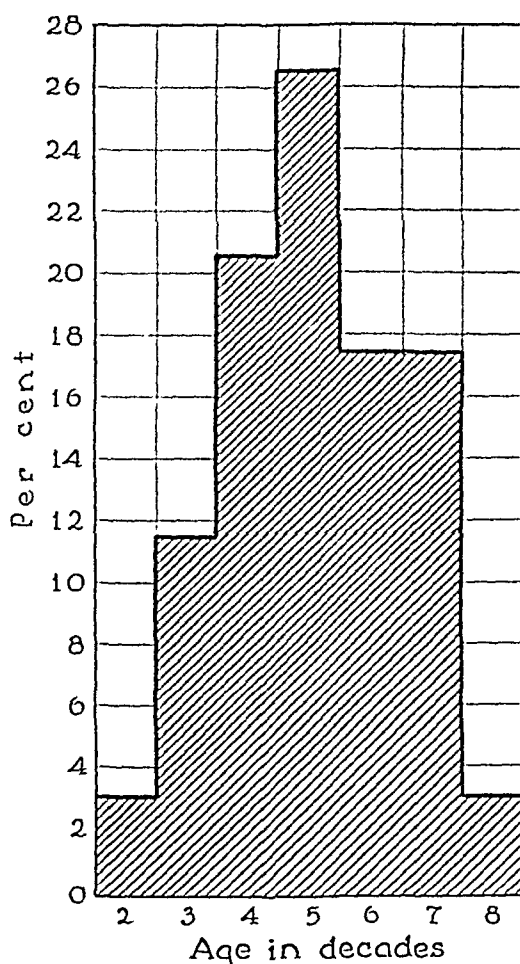


Fig. 3.—Age incidence of calcified nodular aortic deformity associated with mitral lesion (34 cases).

people, 1 per cent in the second decade, 7.5 per cent in the third decade and 18 per cent in the fourth decade, or 47 per cent in the first five decades. It is strongly suggested that the reason old persons so commonly have the aortic calcified type of lesion is because persons having the aortic valve deformity as the chief valvular lesion can live longer, because their hearts can compensate longer, than when the main lesion is on the mitral valve.

The condition of the arch of the aorta strongly suggests that the calcified nodular condition of the aortic valves, in most cases, begins

rather early in life. The smoothness of the intima is a conspicuous finding in at least 90 per cent of the cases. It is apparent that these deformed valves in some way offer a protection to the aorta and lessen the chance of the development of arteriosclerosis. Since the degree of senile arteriosclerosis of the aorta is decidedly less in persons with calcified aortic valve deformities than in persons of a similar age without them, it seems evident that the injury to the aortic cusps begins in most cases early in life. The fact that the calcified nodular aortic type of lesion is common in old persons cannot be used as evidence to indicate that such aortic lesions are due to metabolic changes that occur chiefly in old persons.

An objection sometimes offered to the theory of infection in the etiology of calcified nodular aortic valve deformities is that the evidence of acute rheumatic fever in the group is slight. It is therefore necessary to inquire concerning the frequency of rheumatism in these cases. Three things indicative of previous attacks of acute rheumatic fever are a positive history of rheumatism, evidence of nonspecific pericarditis (acute or healed) at autopsy and the association of rheumatic lesions on one or more of the other valves. Rheumatism, as indicated by a positive history or by evidence of a previous pericarditis, or by both, was present in 49 per cent of the 160 cases of the noncalcified nodular type of old valve deformity and in 40 per cent of the 93 cases of the calcified nodular valve deformity, or nearly as frequently in the latter as in the accepted old rheumatic cases. No group of noncardiac cases coming to autopsy would have as high an incidence of adherent pericardium as is found in this group of cases of calcified nodular aortic valve deformity.

The association of rheumatic lesions, acute or healed, in one or more of the other valves also suggests that the calcified nodular aortic valve deformity, as well as the deformities on mitral, tricuspid or pulmonary valves, may be due to infection with the rheumatic virus. Four of the persons with calcified nodular aortic valves had acute rheumatic vegetations on the mitral valve. There were healed lesions in the mitral valve in 34 of the 93 persons with calcified nodular aortic valves. Three not included in this series of 93 had bacterial vegetations engrafted on the calcified nodular aortic valves.

The frequent evidence of previous attacks of rheumatic fever in the group with the calcified nodular valves would seem to support the infectious rather than the metabolic nature of the valve changes, especially since the incidence of rheumatic fever is practically as great as in the group in which the rheumatic virus is regularly accepted as the etiologic agent.

#### GROSS STRUCTURES OF THE VALVES

Another approach toward information concerning the etiology of these calcified nodular aortic valve deformities is to study the gross

appearance of the valves. An estimation of the degree of diffuse thickening of the cusps is important. Sixty-eight of the 93 hearts were preserved and were available for careful study. The degree of diffuse thickening in the cusps in these 68 hearts is shown in table 2.

It is to be observed that all but 2 of the 68 hearts showed some degree of diffuse thickening of the cusps. In about 80 per cent there was a severe degree of thickening. Typical arteriosclerotic changes were commonly found in all the valves, especially in the mitral; but thickening of the cusps was not associated with the arteriosclerotic changes. On the other hand, repeated attacks of rheumatic valvulitis definitely bring about thickening. It seems reasonable to assume that the diffuse thickening in the cusps in the calcified nodular type of valves is most probably due also to attacks of rheumatic valvulitis, since the calcified nodular valve deformity is the outstanding type when the chief involvement is in the aortic valve.

TABLE 2.—*Degree of Diffuse Thickening of the Cusps in Calcified Nodular Aortic Valves in Sixty-Eight Cases*

Degree	Cases	
	Number	Per Cent
0	2	3.0
+	12	17.5
++	25	37.0
+++	29	42.5
Total.....	68	100.0

Grade + indicates a relatively small amount of thickening. Grade +++ is an extreme degree of thickening, and grade ++ is intermediate.

Another gross anatomic consideration of importance in studying the etiology of valve deformities is whether the cusps are fused one to the other. In the 68 hearts examined, fusion of the cusps from a slight to an extreme extent was present in 88 per cent. It was often extensive enough to cause severe stenosis. This fusion suggested an inflammatory rather than a metabolic basis for the changes in the valves.

The location of the calcified nodules in the cusps is considered significant by some observers. Mönckeberg<sup>2</sup> emphasized the fact that in his cases the nodules were on the aortic surface of the cusps. This suggested to him that the process was carried over from the aorta, because the fibrous layer of the cusps on the aortic side was a continuous structure with the aorta. In our cases, the relative frequency of the calcified nodules on the aortic side alone, on the ventricular side of the cusps alone; or on both sides was as shown in table 3.

It is seen that the frequency of nodules on the aortic surface alone was greater than on the ventricular surface alone, but neither condition was common. A greater frequency on the aortic surface would be likely, regardless of the etiologic factor, since the aortic half of the cusp consists of a denser structure and vessels are to be seen in this half

much less frequently. Calcification occurs in tissues poor in blood supply. The frequency of the nodules existing within the cusps and extending outward so as to be evident on both sides is high, 88 per cent. The location of the calcified nodules in the thickened cusps does not seem in any way to support the arteriosclerotic basis for the etiology of the valve deformities as suggested by Mönckeberg.<sup>2</sup> The location of the nodules in the thickened cusps, on the other hand, fits in with an inflammatory process with scar formation that becomes calcified.

The location of the calcified nodules in relation to the angle joining the aorta and the cusps was considered important by Mönckeberg. He believed that the angle was the usual location of the nodules and thought that the nodule was continuous with the arteriosclerotic process in the aorta. Acute rheumatic vegetations are generally found on the ventricular surface of the cusps at about one third the distance from the free margin to the base of the cusps. In the cases of calcified nodular aortic valve deformity that I studied, the nodules were in the angle in 23 per cent. In the remaining 77 per cent of the cases, they were

TABLE 3.—*Location of Calcified Nodules on Aortic Cusps in Sixty-Eight Cases*

Aortic Surface Alone		Ventricular Surface Alone		Both Surfaces	
Number	Per Cent	Number	Per Cent	Number	Per Cent
7	10	1	1.5	60	88

located chiefly in the level that is most frequently attacked by acute rheumatic vegetations. The location of the calcified nodules on the aortic cusps seems to favor an infectious rather than a metabolic process as the etiologic factor in producing the calcified nodular type of valve deformity.

Nothing in the gross structure of the valves seems to indicate an arteriosclerotic or a metabolic basis for the anatomic change. On the other hand, all changes noted are what might be expected in a healed scarred area due to inflammation. The gross appearance of the calcified nodular aortic valves does not seem to bear any relation to arteriosclerosis, but it does bear a definite resemblance to the gross structure of calcified mitral valve deformities, which are generally accepted as being the result of rheumatic infection.

#### MICROSCOPIC STRUCTURE OF THE VALVES

The calcified nodular valves were studied microscopically, and the structure was compared with that of valves the defects of which were known to be of rheumatic origin. In a previous study, Clawson and Bell<sup>4</sup> found the microscopic appearance in old rheumatic valves to vary from stages in which the polyblastic cellular reaction was almost as active as in acute rheumatic valvulitis, to those in which practically nothing was left but a hyalinized scar. A polyblastic cellular reaction



in which varying numbers of mononuclear and polynucleated polyblasts with various degrees of activity are found is a definite indication of an inflammatory process. The calcified nodular valves in 35 cases were studied microscopically. In 22 (63 per cent) there was a definite polyblastic type of reaction. In 13 (37 per cent) the structure consisted of a scar without evidence of an active inflammation. This relative proportion of active polyblastic reactions to definitely healed scars is practically the same as that found in old rheumatic mitral valves. The polyblastic reaction seems evidently not to be a foreign body reaction, due to the presence of calcium in these valves, for the reaction bears no definite relation to the position of the calcium. The reaction may be pronounced in the absence of calcium or there may be large deposits of calcium without any polyblastic cellular reaction.

One of the most conspicuous things noted in a healed scarred old rheumatic valve is the presence of blood vessels. Blood vessels are not seen in normal aortic cusps by microscopic examination. Vessels easily seen with the microscope develop in old rheumatic scarred valves evidently as a result of inflammation. Thirty-five valves of the calcified nodular type were examined microscopically for the presence of blood vessels. In 32 (91.5 per cent) blood vessels large enough to be seen easily with the low power lens were present. These blood vessels, like the polyblastic cellular reaction, bore no relation to the position of the calcium. The presence of bone was not uncommon. This appeared to be a result of inflammation. Osseous tissue is not infrequently encountered in healed pulmonary tuberculous lesions. Cholesterol crystals, so conspicuous in the intima of arteries in arteriosclerosis, were not found in any of the 35 valves examined.

The microscopic structure of the calcified nodular aortic valves was similar to that of valves with defects known to be due to rheumatic inflammation. There was no resemblance to the structure of arteries with arteriosclerosis. The changes seemed evidently to be due to inflammation of rheumatic origin and not to a metabolic or to an arteriosclerotic process.

#### COMMENT

Ninety-three cases of calcified nodular aortic valve deformity were compared with 160 cases of noncalcified nodular valve deformity. The purpose of this comparison was to obtain information in respect to the frequency, the etiology and the pathogenesis of the calcified nodular aortic valve deformity (the so-called arteriosclerotic valve).

The calcified nodular type of aortic valve deformity is of common occurrence. In fact, this type is the usual one on the aortic cusps. Stenosis of the aortic ring is frequently found. Clinically a diagnosis of aortic stenosis should be made. If it is not, a high percentage of old valve deformities will not be properly diagnosed.

The time at which death occurs in this group does not seem to be of any significance in respect to the etiology of the process. While it is true that death occurs at a later age in persons having the calcified nodular aortic valve deformity than in those having healed mitral lesions, yet it is to be observed that nearly half of such persons die by the time that they are 50 years old. The reason that this type of valve lesion is more common in old persons than the noncalcified nodular type seems to be explained by the fact that a heart with an aortic lesion will compensate longer than a heart with a mitral deformity, or with an aortic and a mitral deformity, and by the fact that aortic valve deformities of severe involvement are generally of the calcified nodular type.

The gross findings in these valves, such as diffuse thickening and fusion of the cusps, and the location of the calcified nodules in the cusps, all tend to support the inflammatory rather than the metabolic basis for the changes in the valves. Likewise, the microscopic structures, as the polyblastic cellular reaction, the presence of blood vessels within the injured cusps and the absence of cholesterol crystals, strongly support the infectious basis in the etiology of the calcified nodular aortic valve deformities. There does not seem to be any indication for considering the changes in the valves as due to a metabolic process such as arteriosclerosis.

#### SUMMARY

Aortic valve deformities of severe involvement are usually of the calcified nodular type.

An aortic stenosis of severe grade is a common finding with this type of valve deformity. Clinically, a diagnosis of aortic stenosis should frequently be made.

The reason the calcified nodular type of aortic valvular deformity is so common in older people seems to be that the heart will compensate better with an aortic deformity than with a mitral deformity and most severe aortic valve deformities are of the calcified nodular type.

The frequency of rheumatism in these cases, as indicated by a previous positive history, by the presence of an adherent pericardium or by an association of deformities in other valves, strongly suggests an infectious basis for the calcified nodular valve deformity.

The gross and microscopic structure of these calcified nodular aortic valves indicates that the process is infectious.

There seems to be no support for the metabolic theory of the structural changes in these valves.

It is doubtful whether a valve deformity severe enough to cause cardiac insufficiency is ever due to a metabolic disturbance such as arteriosclerosis.

The term arteriosclerotic valve deformity should not be used in describing valvular insufficiency or stenosis.

# HOMOLOGOUS LIVER AS A STIMULUS TO HEPATIC REGENERATION \*

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AND

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A normal regenerative capacity is often demonstrable in both spontaneous and experimental lesions of various parenchymal organs. That control is exercised by the body as a whole is shown by the constant ratio of body weight to organ-weights. The stimulus that initiates the regeneration and the control mechanism that stops it are often imperfectly understood. In neoplastic growth the tumor cells are not under the complete control of the regulatory mechanism that limits proliferation. Once the blastoma has arisen the failure of the host to limit its growth is apparently due to a defect in the tumor cell, since successive hosts behave similarly to the transplanted tumor. The exact character of the defect and its method of origin are not known. Although various factors playing rôles in blastomatous and nonblastomatous growth are known, the ultimate mitotic stimulus and its reactions within the cell are unknown. Our experiments were made to test the effect of the lytic products of a cell on its regeneration.

Much information has been obtained in regard to the stimulant action of various tissue derivatives on cells proliferating in vitro. Carrel and Baker <sup>1</sup> found that the regeneration of skin of the guinea-pig was much accelerated by the addition of proteose and other primary derivatives of protein to the tissue culture mediums. To these investigators especially is due the knowledge that polypeptides stimulate cell growth. The addition of other substances, such as vitamins, to cultures of sarcoma tissue caused no acceleration of growth. Carrel <sup>2</sup> stated that growth-promoting substances of the general nature of polypeptides are present in the various organs, and that they may be manufactured by leukocytes from fibrin and cell débris in inflammatory processes and wounds. Hammet <sup>3</sup> has extensively investigated the stimulant effect of compounds containing the sulphydryl

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\* From the Department of Pathology, Loyola University School of Medicine.

1. Carrel, A., and Baker, L. E.: *J. Exper. Med.* **44**:503, 1926. Baker, L. E., and Carrel, A.: *ibid.* **47**:353, 1928.

2. Carrel, A.: *Proc. Inst. Med., Chicago* **8**:62, 1930.

3. Hammet, F. S.: *Protoplasm* **7**:20, 1929.

group, and is of the opinion that this chemical group alone is active in the promotion of growth. Voegtlin and Chalkley<sup>4</sup> added glutathione, which is a tripeptide containing the sulphydryl group to a nonnutrient medium of *Amoeba proteus* and observed an increase in the rate of proliferation. Such findings suggest that the stimulus for cell regeneration is of a chemical nature. Gurwitsch<sup>5</sup> recently advanced the theory that cell division is stimulated by ultraviolet rays given off by living cells.

Within the body, the cells are evidently in an environment more complex than in the tissue culture. The plasma in which the body cells are bathed may contain not only growth-promoting substances, but also inhibitory agents active in the regulation of cell growth. In fact Carrel<sup>2</sup> recognized an inhibitory effect of plasma that increased with the age of the animal. In spite of this complex situation, the composition of the plasma may be varied by the enteric or parenteric administration of various substances. This Loeb<sup>6</sup> performed in the case of the thyroid gland by feeding guinea-pigs subjected to partial thyroidectomy with thyroid gland. The result was that the regenerative activity of the thyroid remnant was inhibited. Potassium iodide, on the other hand, not only failed to inhibit compensatory hypertrophy, but actually stimulated it.<sup>7</sup> Although small amounts of protein may pass through the intestinal wall unchanged, most of it is subjected to extensive lytic changes, and only the less complex compounds, such as thyroxine, reach the blood stream. In our experiments, parenteric administration was employed in order to eliminate the digestive action of the gastrointestinal tract. The experiments may be divided into two general groups, the first consisting of partial hepatectomies and ligations and the second of intraperitoneal injections of crushed liver into animals otherwise normal. The white rats used in all of the experiments were obtained originally from the Wistar Institute, and during the past several years had been inbred to a considerable extent. All animals were kept on the same adequate uniform diet.

#### REGENERATION OF HEPATIC CELLS AFTER HEPATECTOMY AND AFTER LIGATION OF THE LIVER

The operative procedure for both the partial removals and the ligations was simple. A 5 cm. length of linen tape, 4 mm. wide, was folded, and the middle portion pushed up beneath the diaphragm and the uppermost of the three left lobes. One of the free ends of the ligature was then carried toward the left, encircling the lobes to be

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4. Voegtlin, C., and Chalkley, H. W.: Pub. Health Rep. **45**:3041, 1930.

5. Gurwitsch, A.: Arch. f. Entwcklngsmechn. d. Organ. **100**:11, 1923.

6. Loeb, L.: J. M. Research **41**:481, 1920.

7. Loeb, L.: J. M. Research **40**:199, 1919.

ligated, and the other end was pressed into the large fissure separating these lobes from the remainder of the liver. The ligature was then drawn tight and tied. In this way, the circulation to the three left lobes was shut off. In the hepatectomies, the ligated lobes were cut off close to the ligature. The total mortality at first reached 20 per cent, but later was decreased somewhat with some variation according to subsequent procedures. Usually when the animals were alive and doing well on the third day, they continued so. The restoration of the liver to its normal weight after partial hepatectomy is quite well understood. Higgins and Anderson<sup>8</sup> and Higgins and Priestley<sup>9</sup> found that the liver was restored to its normal weight in about three weeks after the removal of from 65 to 75 per cent. These investigators determined the actual weight of the regenerating liver at stated periods after the hepatectomy. The weight is influenced by the blood content and the amount of other cellular elements, especially of reticulo-endothelium and erythroblasts. Since we were especially interested in the hepatic cells, their mitotic activity was determined directly. At the termination of the experiment the entire liver was fixed in 10 per cent formaldehyde, and blocks were cut at right angles to the large surfaces so as to include both the thick diaphragmatic portion and the thin margins. With the mechanical stage, all mitoses in two or three sections were enumerated, and the average per section was set down in tables.

Not only were mitoses absent in animals that died from the experimental procedures, but they were usually absent in animals killed while obviously sick. In the dog, autolytic liver has been found to be highly toxic, with fatal results following the intraperitoneal injection of 100 Gm. Andrews and Hrdina<sup>10</sup> made a careful study of the fatal peritonitis produced by injections of liver, and reviewed the work of the earlier investigators of the lesion. The peritonitis consisted of an outpouring of sanguineous fluid containing little pus and little fibrin, which was associated with an overwhelming gas bacillus infection. They interpreted the fatal peritonitis as the result of some toxic agent contained in both autolyzed and fresh liver that created a great increase in the permeability of the bowel to *Bacillus welchii* and a rapid infection of the liver. They found the toxic factor to be water-soluble and thermostabile and regarded it as a proteose or a peptone. Apparently the rat is much less susceptible to invasion by *Bacillus welchii*, but in many of our dead animals an appearance in the peritoneal cavity identical

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8. Higgins, G. M., and Anderson, R. M.: Proc. Staff Meet., Mayo Clin. **5**:243, 1930.

9. Higgins, G. M., and Priestley, J. T.: Proc. Staff Meet., Mayo Clin. **6**:249, 1931.

10. Andrews, E., and Hrdina, Leo: Surg., Gynec. & Obst. **52**:61, 1931.

with that described in the dog was found. Had we found a decrease in regeneration in the animals that received liver, the results would not have been easy to interpret, on account of the possible toxic effect of the peritonitis.

The weight of the three left lobes represented from 30 to 45 per cent of the total weight of the liver. On the first and second days after the removal of this amount of liver few mitoses were found. After this time, regeneration developed and continued moderately active for two weeks (table 1). With the exception of rat 128, none of the rats presented numerous mitoses. The large number of mitoses in rat 128 was not explained. For the eleven animals in this group the average number of mitoses was 5.3 per section, as compared with an average of 0.04 for thirty-two normal rats (table 2). In our normal rats relatively few mitoses were present. In rats weighing under 150 Gm. (table 2,

TABLE 1.—*Cell Mitoses in Livers of Rats Subjected to Hepatectomy*

Rat	Weight, Gm.	Days Following Hepatectomy	Mitoses*
104.....	120	7	1.0
106.....	125	3	0.0
113.....	150	4	0.0
115.....	230	5	0.0
118.....	200	9	0.5
125.....	145	3	1.5
127.....	180	6	1.0
128.....	210	9	45.5
129.....	265	9	8.0
130.....	365	2	0.5
131.....	215	9	0.5

\* Average number in sections.

nos. 142, 143, 148, 147, 149, 150, 187, 191, 192, 193, 194 and 195), 1 or 2 mitoses were found; the largest number was 5. In a majority of the normal rats no mitoses were present in the two or three sections examined. The experiments show that the removal of about one third of the liver is followed, after the first three or four days, by a mitotic activity greater than that seen in normal, young, actively growing rats. Early in the work the effect of local trauma on the liver was studied by snipping small bits from the edge and surface of the liver (first twelve rats in table 2). In rat 24, 1 or 2 mitoses per section were found, but these were not in the vicinity of the wound. The hepatic cells manifested no tendency to regenerate, when injured locally, and in this respect they differed from the epithelia of the skin and mucous membrane surfaces.

The ligations were identical with the partial hepatectomies, except that the ligated lobes were not removed. A small amount of blood passed through the ligature, since certain structures, including some of the bile ducts, remained alive in the periportal tissue. However, prac-

tically all of the ligated liver was necrotic at the end of twenty-four hours and at the end of two days showed distinct evidence of solution. In from three to five days, the trabeculae of the hepatic cells were largely dissolved. Since there were no gross breaks on the surfaces of the ligated lobes, it seemed likely that the dissolved substances passed into the general circulation through the blood vessels not completely closed by the ligature. In this way, the dissolved liver entered the general circulation and was carried to the intact portions. In the thirteen rats

TABLE 2.—*Cell Mitoses in Livers of Rats Subjected to Local Snipping of Hepatic Tissue and in Livers of Normal Rats*

Rat	Weight, Gm.	Days Following Local Injury to Liver	Mitoses*	Comment
19.	198	2	0.0	Snip
21.	186	1	0.0	Snip
24.	134	3	1.0	Snip
29.	100	1	0.0	Snip
37.	94	1	0.0	Snip
46.	96	1	0.0	Snip
61.	166	2	0.0	Snip
62.	262	3	0.0	Snip
63.	106	3	0.0	Snip
65.	210	4	0.0	Snip
66.	240	2	0.0	Snip
68.	240	4	0.0	Snip
142.	94	..	0.0	Normal
143.	118	..	0.0	Normal
148.	150	..	2.0	Normal
147.	140	..	2.0	Normal
149.	100	..	5.0	Normal
150.	110	..	0.5	Normal
181.	220	..	0.5	Normal
182.	200	..	0.0	Normal
184.	190	..	0.0	Normal
185.	260	..	0.0	Normal
186.	180	..	0.0	Normal
187.	150	..	0.0	Normal
188.	160	..	0.0	Normal
189.	160	..	0.0	Normal
190.	100	..	0.0	Normal
191.	99	..	0.0	Normal
192.	114	..	1.0	Normal
193.	112	..	0.0	Normal
194.	108	..	0.5	Normal
195.	105	..	0.0	Normal

\*Average number in sections.

(table 3) the average number of mitoses per section was 11.1 as compared with 5.3 in the rats with hepatectomies. Perhaps the most noticeable difference seen in a comparison of tables 1 and 3 is the regular presence of 3 or more mitoses from the third to the fourteenth day in the rats with ligations.

#### EFFECT OF INJECTIONS OF HOMOLOGOUS LIVER ON REGENERATION OF HEPATIC CELLS AFTER HEPATECTOMY

The method of preparing the liver for injection through a large, short needle with a lumen 1.5 mm. in diameter was to remove the entire liver aseptically and crush it with a pestle in a sterile mortar. Some-

times the addition of a few drops of distilled water was necessary to bring the macerated mass to the proper consistency for injection. The hepatectomy was performed by the method described. On the second day, the macerated liver was injected intraperitoneally into all the rats (except rat 169), and into four of the rats a second dose was injected. The results, shown in table 4, were the most decisive obtained. With the exception of a heavy older rat (no. 169), all the rats showed the hepatic

TABLE 3.—*Cell Mitoses in Ligated Livers of Rats*

Rat	Weight, Gm.	Days Following Ligation	Mitoses*
108.....	140	3	7.0
110.....	212	5	4.0
111.....	225	20	0.0
112.....	170	14	3.0
120.....	190	4	7.0
121.....	190	1	0.0
122.....	210	6	38.0
123.....	190	7	26.0
124.....	190	7	3.0
135.....	200	8	5.0
138.....	215	9	27.0
141.....	203	6	21.5
144.....	224	28	3.0

\* Average number in sections.

TABLE 4.—*Cell Mitoses in Livers of Rats Subjected to Hepatectomy and Treated by Injection of Homologous Liver*

Rat	Weight, Gm.	Days Following Hepatectomy	Amount Macerated Liver Injected, Gm.	Mitoses*
155.....	210	5	7.0 (2nd day)	7.0
156.....	225	5	7.0 (2nd day)	38.0
157.....	235	7	7.0 (2nd day)	5.5
158.....	150	7	7.0 (2nd day)	64.0
160.....	150	8	12.5 (2nd and 6th days)	205.0
162.....	182	9	6.5 (6th day)	344.0
163.....	190	9	12.5 (2nd and 6th days)	156.0
169.....	380	11	15.5 (5th and 7th days)	0.0
172.....	220	12	15.5 (2nd and 8th days)	297.0

\* Average number in sections.

cells actively proliferating, and in the nine rats the average number of mitoses was 124 per section. In none of the rats with simple hepatectomies or ligations did the number reach 100. In single fields seen under the high power dry lens, more mitoses were usually present than in entire sections of the livers of rats not treated by injection of liver, from which the same percentage of liver had been removed (table 1). In some fields almost one-half the hepatic cells were in karyorrhexis. So far as could be determined from the relatively small number of animals in the series, the hepatectomized rats withstood the injections approximately as well as the nonhepatectomized normal rats of the same weights.



EFFECT OF INJECTIONS OF HOMOLOGOUS LIVER ON HEPATIC CELLS  
IN NORMAL RATS

An attempt was made to induce proliferation in the hepatic cells of normal rats by injections of liver. When 6 Gm. or more of crushed liver was injected as a single dose into the peritoneal cavities of rats weighing from 150 to 200 Gm., a considerable mortality resulted from the serosanguineous peritonitis. In general, the normal rat tolerated the large doses scarcely as well as the hepatectomized animals. In rats weighing about 200 Gm. that were given an injection of 6 Gm. of liver, mitoses usually were not found (table 5). In five rats weighing under 175 Gm. each of which had received two doses of 6 Gm. or more, mitoses were found. In normal, untreated rats of this weight, mitoses were usually not found in the routine examination of two sections.

TABLE 5.—*Cell Mitoses in Livers of Normal Rats Treated by Injection of Liver*

Rat	Weight, Gm.	Duration of Observation, Days	Amount Macerated Liver Injected, Gm.	Mitoses*
151.....	132	7	12.0 (1st and 4th days)	3.5
152.....	150	8	12.0 (1st and 4th days)	8.3
154.....	164	7	14.0 (1st and 4th days)	23.3
174.....	195	9	15.0 (1st and 5th days)	5.0
176.....	170	7	13.5 (1st and 4th days)	1.0
177.....	200	4	6.0 (1st day)	0.0
179.....	200	7	6.0 (1st day)	....
180.....	325	4	7.5 (1st day)	0.0

\* Average number in sections.

## COMMENT

The view is prevalent that necrotic tissue even in the absence of bacterial invasion is toxic. The local leukocytic reaction about necrotic tissue and certain systemic reactions, such as elevation of temperature following infarction, are cited as toxic manifestations. The data obtained by tissue culture methods cast doubt on this conception. Carrel and Baker<sup>11</sup> showed conclusively that the products of proteolysis, such as the polypeptides, are growth-promoting, although the individual amino-acids do not stimulate growth. In the body, where conditions are less simple, few tissue products have been tested to determine their effect on specific types of living cells. Particulate matter, as shown by the intraperitoneal injection of suspensoids and also coarser suspensions, such as that of red blood corpuscles, quickly passes from the peritoneal cavity into the lymphatics and may reach the blood stream. Foreign protein, of course, readily enters the blood from the peritoneal cavity. If one may judge from the autolytic changes observed microscopically in the injected liver, the protoplasm of injected hepatic cells reaches the

11. Carrel and Baker (footnote 1, first reference).

liver of the host in solution by way of the general circulation. McJunkin and Matsui,<sup>12</sup> investigating the local action of dead macerated epidermis on the regeneration of epidermis, found that it stimulated this regeneration, and that the stimulant effect was somewhat specific, since it did not follow the application of macerated liver.

It seems to us that the chief environmental difference between cells proliferating *in vitro* and those proliferating *in vivo* is the contact of the latter with a constantly changing plasma. This makes possible a continuous local removal by the cells of a stimulating or an inhibiting substance supplied to the circulating blood elsewhere. In our experiments, the dissolved protoplasm of a particular type of cell plus any disintegration products formed from such cells was added to the plasma, and within the liver it stimulated mitosis. The substance presumably acts as a chemical stimulus or as a physicochemical excitant. The current explanation offered for regeneration of parenchymal tissue is that of physiologic need or functional necessity. It is not apparent that the macerated tissues increase the functional requirements. It is more plausible to view the formative stimulus as a food element. Relative differences between types of cells are apparent. In the skin there is an immediate proliferative response to correct any loss of epidermal cells. Carrel<sup>2</sup> held that substances such as those present in the embryo and among the decomposition products of fibrin and dead tissue are directly produced by the injury and by the subsequent action of enzymes, and that these substances stimulate cells to regenerate. Unlike the epidermal cells the hepatic cells do not regenerate in response to local injury (twelve rats in table 2). This difference in regenerative activity may be inherent in the cells, but there is an obvious difference in environment. Granulation tissue forms in the hepatic wound, but its vascularity does not equal the efficient normal sinusoidal blood supply. In cutaneous wounds, the granulation tissue delivers an excess of blood as compared with the normal dermis. Here again a pabulum theory may be employed to explain the results, although it offers no ultimate explanation for cell division. In cellular metabolism specific cell products are constructed from the general food supply. Based on our experiments, certain of these products may be recognized as specific food substances. The plasma may contain these specific products, perhaps only in traces, as well as the general nutritive substances. Higgins and Priestley<sup>9</sup> emphasized the importance of increased blood in the regeneration following partial hepatectomy. An increase in blood supply would deliver to the epidermoid or to the hepatic cells not only additional general nutrition, but also added amounts of any "type-cell food" present in the

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12. McJunkin, F. A., and Matsui, T.: Effect of Homologous Macerated Skin on the Regeneration of Epidermis, *Arch. Path.* **12**:794, 1931.

plasma. With a relative increase of the latter in the plasma, the proliferation of cells might be stimulated without an increase in the blood supply. The greatly increased rate of regeneration in the hepatectomized animals treated by injection of liver over that in animals with simple hepatectomies is best explained by a qualitative rather than a quantitative change in the blood, since injections of macerated liver do not appear to increase the flow of blood through the liver. In the destructive lesions of parenchymal organs often associated with regeneration, products from the necrotic parenchyma enter the circulation. If it is assumed that the various "type-cell foods" are present in traces in the circulation, a decrease in the total amount of such tissue by extirpation would make available a relatively greater amount of this specific nutrient for the cells in the remnant of the gland. The formative stimuli appear not to be of the nature of glandular secretions or hormones. Indeed, the experiments of Loeb<sup>6</sup> with thyroid feeding suggest that such products may be inhibitory. Finally, it must be kept in mind that present knowledge does not warrant the assumption that cell regeneration and its control or lack of control rests entirely on the supply of nutritive elements, general and specific, or on the exhaustion of this supply.

#### CONCLUSIONS

In the rat subjected to partial hepatectomy, injections of homologous macerated liver greatly increase the regeneration of hepatic cells.

Absorption of the rat's own ligated, necrotic liver leads to some increase of karyorrhexis in the remnant.

Maximum parenteral injections of homologous liver into half-grown rats stimulate somewhat the proliferative activity of the liver.

The dissolved protoplasm of cells (hepatic) contains a type-cell formative stimulant.

# ASBESTOSIS

REPORT OF TWO CASES \*

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Asbestos as such was known to Pliny. Charlemagne is said to have possessed a table cloth made of it, which was cleansed by passing through fire. The mineral deposits of asbestos are found in Canada, Italy, Africa and Rhodesia. It is a silicate occurring in minerals in combination with iron, copper, calcium or magnesium. It occurs in the form of fibers which can be woven. In addition, finer particles are used in the manufacture of sheeting, tiles, asbestos millboard and paper. The increasing need for brake linings for motor cars, fireproof curtains, insulations, mattresses and steam packings has expanded the spinning industry considerably. The use of the dust in cement and plaster for the erection of fireproof buildings has increased the danger of inhalation in the most hazardous form of the industry. Its expansion from 500 tons in 1880 to 330,000 tons in 1925 and the fact that methods of manufacture have been introduced for utilizing the fine dust for new purposes have contributed to the beginning appearance of a pneumokoniosis due to inhalation of asbestos dust,<sup>1</sup> known as pulmonary asbestosis.

In 1906, Montague Murray<sup>2</sup> presented evidence before the Departmental Committee on Compensation for Industrial Diseases, that he had had under his care in 1900 a worker employed in an asbestos factory who developed respiratory symptoms and died. At the autopsy, extensive pulmonary fibrosis was found, which Murray attributed to inhalation of asbestos dust.

In 1924, eighteen years later, Cooke<sup>3</sup> reported a similar case complicated by pulmonary tuberculosis. He was the first to describe the

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1. Merewether, E. R. A.: *J. Indust. Hyg.* **12**:198, 1930.

2. Murray, Montague: Departmental Committee on Compensation for Industrial Diseases, Minutes of Evidence, Appendices and Index, 1907, Cd. 3496, p. 127; Report 1907, Cd. 3495, p. 14.

3. Cooke, W. E.: *Brit. M. J.* **2**:147, 1924; **2**:1024, 1927.

"curious bodies" in the lung now known as "asbestosis bodies," while McDonald<sup>4</sup> worked out the histologic features. The latter believed that the bodies were portions of asbestos in process of alteration and absorption by hydrolysis, with the passing of silica into a colloidal state and later a gel. Stewart and Stewart and Haddow<sup>5</sup> showed that these bodies could be found in the juices expressed from affected lungs and could be found in the sputum by digesting it with equal quantities of antiformin. Lynch and Smith,<sup>6</sup> working without knowledge of this, demonstrated these bodies by digesting sputum with 10 per cent sodium hydroxide. Gloyne<sup>7</sup> proved by means of dark-ground illumination, that when the golden-yellow asbestosis body was dissolved in concentrated sulphuric acid, it had a central core consisting of a minute asbestos fiber. The asbestos fiber itself under dark-ground illumination has the appearance of a sharp piece of wire. Fibers measuring 200 microns<sup>8</sup> can pass the protective mechanism of the upper respiratory tract and enter the lung.

#### REPORT OF CASES

CASE 1.—J. M., a white man, entered the Jefferson Medical College Hospital on June 8, 1931, in the service of Dr. Edward Klopp. He complained of headache, abdominal distention and eructation of acid and gas. A diagnosis of chronic cholecystitis was made. A cholecystectomy was performed. Peritonitis developed, and the patient died. The history disclosed certain facts relative to asbestosis. The patient had worked as a spinner in an asbestos mill for nine years. Although the atmosphere was not very dusty, he wore silver dust protectors in his nostrils. For five years following such employment, he worked as a laborer. During the past five years, he had morning cough with moderate expectoration. There was no history of hemoptysis or pain in the chest. He had some dyspnea and cardiac palpitation. Physical examination was unimportant with the exception that he had an emphysematous type of chest. The important roentgen observations were slightly increased peribronchial markings throughout both lungs and some calcific deposits in both root areas. The cardiac shadow appeared to be normal. The sputum was not examined.

At the postmortem examination, the heart appeared normal. The left lung weighed 270 Gm. and the right 320 Gm. They were bluish gray, practically free from adhesions and crepitant throughout. On section, no gross pathologic lesion was demonstrable. Microscopically, there was a moderate grade of emphysema.

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4. McDonald, S.: *Brit. M. J.* **2**:1025, 1927.

5. Stewart, M. J.: *Brit. M. J.* **2**:509, 1928; **2**:581, 1929. Stewart, M. J., and Haddow, A. C.: *J. Path. & Bact.* **31**:172, 1929.

6. Lynch, K. M., and Smith, W. A.: *J. A. M. A.* **95**:659, 1930.

7. Gloyne, S. R.: *Tubercle* **10**:404, 1929.

8. Wood, W. B., and Gloyne, S. R.: *Lancet* **1**:445, 1930.

Some of the alveolar walls were slightly thickened. A few typical asbestosis bodies were seen in the alveolar walls. There were scattered small collections of brown and blackish dust, free and within phagocytic cells, in fibrosed areas. Much of this dust and all of the asbestosis bodies stained positively for iron. The liver and the spleen were not pigmented.

The question of the relation of pulmonary asbestosis to tuberculosis is an interesting one. The fact that they are frequently associated is settled. Whether tuberculosis becomes implanted on pulmonary asbestosis or whether the occupational disease lights up a quiescent lesion is a mooted question.<sup>1</sup> It is true that when tuberculosis occurs, it is liable to be of an anomalous type. Gardner and Cummings<sup>9</sup> exposed series of guinea-pigs to attenuated tubercle bacilli and to an atmosphere laden with asbestos dust and attenuated tubercle bacilli. They found, in the first instance, that normal guinea-pigs receiving the strain of attenuated tubercle bacilli showed tubercles in the lung and in the tracheobronchial lymph nodes comparable to the primary complex in man. The lesions caseated and healed by resolution. Spread of the infection with macroscopic disease in other viscera was rare. On exposing guinea-pigs to attenuated tubercle bacilli and to asbestos dust, 32.2 per cent of one group of animals showed some evidence of spreading tuberculosis. New disease began as a local extension from primary tubercles and metastasized to areas where dust reaction had occurred. The tendency to healing by fibrosis was marked. Macroscopic disease in the spleen was common and occurred occasionally in the liver. In another group receiving asbestos and attenuated tubercle bacilli, the localization of tubercles was atypical. Many bacilli were seen trapped in foci of dust reaction. Some produced local tubercles; others immediately entered dilated lymph vessels and were carried to the tracheobronchial lymph nodes. Tuberculosis of these nodes sometimes occurred without involvement of the lung. Early disease of the spleen and of hepatic lymph nodes occurred in the majority of cases. The combined action of tubercle bacilli and asbestosis in the lung produced more fibrosis than did either agent acting independently.

CASE 2.—J. J., aged 52, entered a hospital in December, 1928. In 1921, he worked in an asbestos factory for a period of about nine months. In this factory, no precautions were taken against inhalation of asbestos dust. Exactly what his duties were was impossible to determine. On admission, he complained of fatigue, loss of weight and a slightly productive cough. Tubercle bacilli were demonstrable in his sputum at that time. On roentgen examination, a cavity in the right upper portion of the chest and a fine stippling in the lower portions of the lower lung

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9. Gardner, L. W., and Cummings, D. E.: *J. Indust. Hyg.* **13**:65, 1931.

fields suggesting pneumokoniosis were found. There was improvement; the patient was discharged and continued under observation as an ambulatory patient. In September, 1930, he was admitted to the Jefferson Hospital for Diseases of the Chest, in the service of Dr. B. Gordon. He complained of the same symptoms as before. Physical examinations revealed the following salient facts: He was markedly emaciated. The chest was emphysematous in type. Expansion was much limited, particularly in the right upper portion, where there was lagging. Over the apex of the right lung, physical signs indicative of a cavity were present. Over the remainder of both lungs, expansion was limited; tactile fremitus was increased; breath sounds were roughened and somewhat exaggerated, and there were numerous scattered râles. The area of cardiac dullness was within normal limits; the heart sounds were distant; there was a mitral murmur at the apex. The abdomen appeared to be normal. There were clubbing of the fingers and curving of the nails; the skin was slightly cyanotic. The patient left the hospital in March, 1931, against medical advice, somewhat improved, but he returned a month later with exacerbation of all his symptoms and an acute respiratory infection. His course was progressively downward; bronchopneumonia developed, and he died, April 2, 1931. At no time during his stay in Jefferson Hospital were tubercle bacilli found in his sputum. There were a moderate secondary anemia, a slight elevation of nonprotein nitrogen, and negative results from Kahn and Wassermann tests. On roentgen examination, Sept. 20, 1931, there was multiple small cavitation in the right upper lobe, and the interlobar pleura below it was much thickened. In the lower lobe, right, and in the middle and lower lobes, left, there was an increase in the markings that resembled a dust change. The heart appeared normal.

At the postmortem examination, the heart weighed 280 Gm. and measured 13 by 8 by 6 cm. The myocardium was mottled red and yellow, and appeared soft and dull. There were many firm, gray, translucent nodules on the free margins of the mitral valve leaflets. The coronaries were thickened and tortuous, and contained atheromatous plaques. The parietal pleura on both sides was thickly studded with small, discrete, gray, pinhead-sized nodules, firm in consistency. Similar ones were present, but much less frequently, on the visceral pleura. Over the bases of both lungs, firm fibrous adhesions completely obliterated the pleural cavities. Between the bases of the lungs and the diaphragm, these adhesions were converted into a thick, tough, yellow, homogeneous substance having the appearance of hyaline cartilage. The right lung weighed 960 Gm. and the left 830 Gm. The consistency of both lungs was much increased over the normal. The bases were particularly firm and tough, and dark brown. The upper half of the right upper lobe was excavated by a large multilocular cavity. The wall of this did not differ in density from that of the adjacent lung tissue, giving the impression that the cavity had formed so rapidly that a limiting fibrous wall was not thrown out. The cavity measured 6 cm. across, and through it coursed many thrombosed blood vessels. It had a foul, fetid odor as of gangrene. Throughout the remainder of this lung there were patchy areas elevated slightly above the cut surface, gray and red, moist, and measuring about 1 cm. in diameter. In the left lung, in addition to what has already been described for it, were two round nodules, similar in appearance, one in the upper portion of the lower lobe and one in the lower portion of the upper lobe on the outer aspect near the pleura. The one in the lower lobe was slightly larger, measuring 4 cm. in diameter. It cut with increased resistance and on section was composed of thick, dense strands of fibrous tissue, intermingled with gray, cheesy material. The regional lymph node draining this area was similar to this nodule. There were no cavities in this lung. In many fresh smears examined from these caseous areas, no tubercle bacilli were found.

Microscopically, the parietal pleura was studded with tubercles. These were composed of epithelioid cells, a few lymphocytes, giant cells and fibrous tissue in considerable amounts. Many were hyalinized to a considerable extent. Caseation necrosis was practically absent. They were of the productive type, with a few well formed tubercles.

The nodules in the left lung were large, caseous areas, divided up into lobules by strands of fibrous tissue. Sections from them and from the tubercles in the pleura were examined for tubercle bacilli, but none were found. In sections taken from the large cavity in the upper lobe of the right lung there were fresh lique-

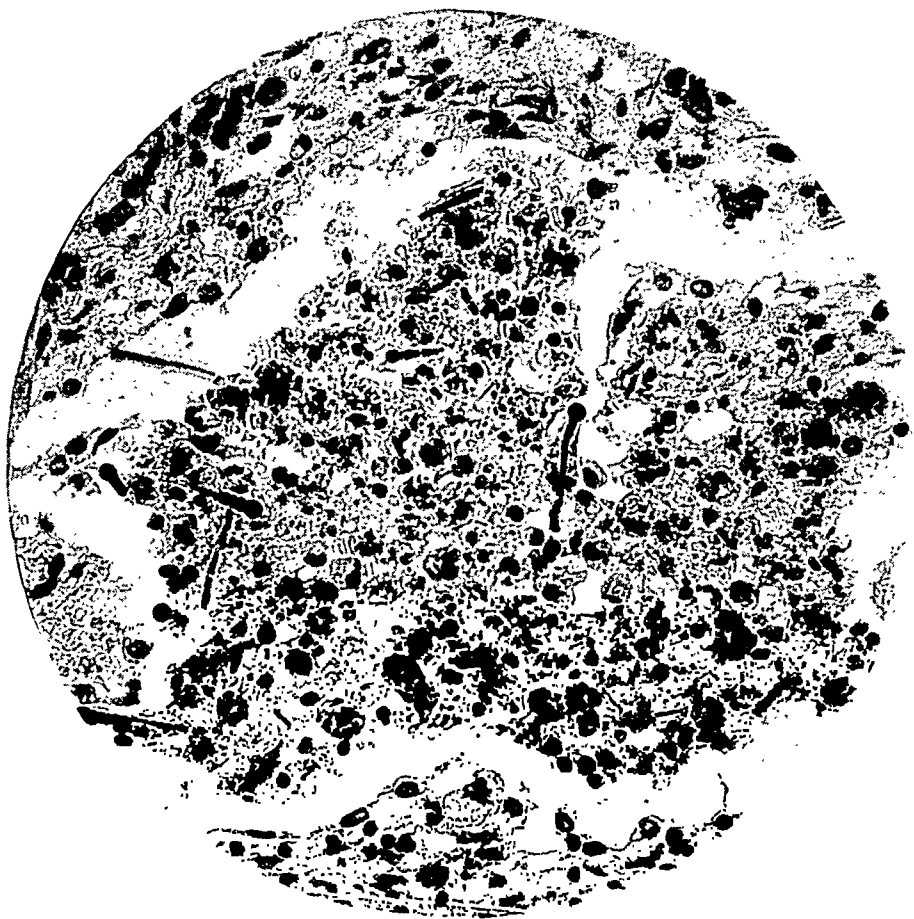


Fig. 1 (case 2).—A small bronchiole completely filled with cellular exudate and "asbestos bodies." The fine segmentation and the clubbed end in some can be distinctly seen;  $\times 400$ .

faction necrosis, very little polymorphonuclear and lymphocytic cell infiltration, little hyperemia and a thin, newly formed layer composed chiefly of young fibroblasts.

The principal lesion of the lungs was a diffuse fibrosis, particularly marked in the lower lobes. In many places, the alveolar walls were markedly thickened; in others, the alveoli had been completely replaced by fibrous tissue. Many bronchi were inflamed, and there were many areas of pneumonia present. A high percentage of the arteries were the seat of an obliterative endarteritis. The most striking feature in the lungs was the "asbestos bodies." They were present in great numbers in the fibrosed areas, in some alveolar walls and spaces and in the



smaller bronchial branches. Particles of them were fragmented and were seen in phagocytic cells. Mainly, they lay loose in small clusters with collections of polymorphonuclear leukocytes and polyblasts about them, often completely occluding a small bronchiole or an alveolar duct. They measured roughly from 10 to 200 microns and were golden brown. They had a variety of appearances, but a characteristic morphology. They were seen often as a series of regular disks very much as are red blood cells in rouleaux formation. Frequently a series of disks ended in a club form on one or on both ends. Sometimes they were seen as long delicate filaments ending in clubbed ends; or again as short clubbed forms, single or double, suggesting a dumbbell. Occasionally, single, paired or coccal forms in chains were found, or even sporelike aggregations. They were present in the juice squeezed from the lungs. They did not stain with hematoxylin-eosin or Gram's stain. They did, however, give the prussian blue reaction for iron in varying degrees of intensity, in the main rather pronounced. In the caseous tuberculous areas in the left lung, these asbestosis bodies were fewer in number than in the adjacent lung tissue. A few were normal, but many were pale with indistinct

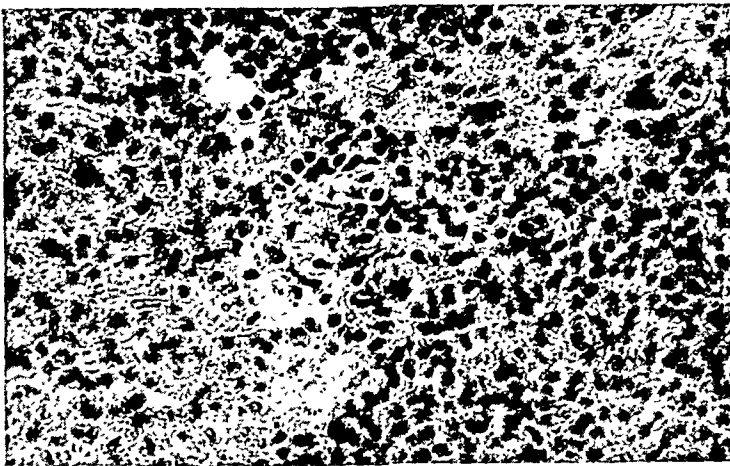


Fig. 2 (case 2).—Spleen. An asbestosis body is lying in the center of the field. One end is clubbed; the other pointed;  $\times 400$ .

outline. When these caseous areas had been stained for prussian blue reaction following the staining of them for acid-fast bacteria (carbol fuchsin saturated solution 95 per cent alcohol, decolorization with 25 per cent nitric acid, Loeffler's methylene blue), the asbestosis bodies stained a pale green instead of a distinct blue, and their morphology was less regular, but they could still be recognized. In addition to the asbestosis bodies there was considerable golden-brown and black, granular pigment present in phagocytic cells. Much of this gave the iron reaction.

In the spleen, the reticulo-endothelial cells were loaded with a fine, amorphous golden-brown and sometimes blackish dust. Some of this dust was extracellular. A few large, typical asbestosis bodies were found. Practically every hepatic cell contained a few granules of a golden-yellow pigment. Many of the Kupffer cells contained considerable quantities of light golden-yellow and blackish granules of pigment. About the portal radicles it was common to see a few cells loaded with pigment. In the mucosa of the stomach there were many phagocytic cells filled with fine, golden-brown pigment granules. In the kidney, many cells were seen in the capillaries of the glomeruli which were filled with coarse granules of a golden-brown and blackish pigment. The lymph node draining the caseous area in

the left lung contained a caseous tuberculous area similar to the one described in that lung, and in addition, many well healed tubercles. All bronchial nodes were deeply pigmented and fibrotic. The pigment was in the form of fine brown and black granules, mainly within phagocytic cells, and a great deal of it stained for iron. A few asbestosis bodies were present.

#### COMMENT

The two cases cited illustrate nicely the extremes and some of the features of the disease. In the first case, as far as the patient's health and life were concerned, the presence of pulmonary asbestosis in the lung was relatively unimportant. He had symptoms doubtfully attributable to it. It was only through the history, which was confirmed by postmortem examination, that the possibility of such a condition was suggested. It is, however, important to point out two considerations in this case. First, it is a question whether the asbestosis had anything to do with his emphysema and cough. As an etiologic factor, the asbestosis cannot be ignored. The other point is that one has to do with preventive medicine. The exposure was for nine years in a comparatively well ventilated factory in which precautionary appliances were used, and yet the patient developed a minor grade of the disease. It would seem that further precautionary means are necessary in this occupation to obviate a disease in itself so preventable.

In the second case, the patient worked in an asbestos factory for a short time, but without a mask. This was long enough to permit his lungs to become densely filled with the dust. Obviously the symptoms did not result from the asbestos per se, but were due to the marked fibrosis resulting from the deposition of the pigment. This demonstrates that the symptoms of a pneumokoniosis may come on years after a short exposure and emphasizes again the absolute necessity for wearing masks in even the less dusty atmospheres.

The marked fibrosis of the pleura, particularly of that interposed between the base of the lung and the diaphragm, is characteristic. The finding of rapidly liquefying areas in the lung is not unusual either, but the presence of such an extensive freshly excavated area is.

We have been able to demonstrate two large tuberculous lesions in the midlung just beneath the pleura, tuberculosis of the regional lymph nodes and tuberculosis of the parietal pleura. The analogy between this form of lesion and that of the tuberculosis of childhood is, to say the least, striking. Gardner and Cummings have shown the course of asbestos inhalation with coincident tuberculous inoculations. Dr. Gardner, in a personal communication to us, replied in the negative to our question as to whether or not any experimental work had been done on the influence of asbestos inhalation on a healed primary focus. It seems to us this would throw light on the subject, for it does not appear that the asbestosis and the tuberculosis were coincidental lesions.

Our second case confirms previous observations that the asbestosis bodies have a tendency to disappear in a caseous area. We have also shown that in such an area following an acid-fast stain (25 per cent nitric acid) the bodies do not stain so well for iron and have a less definite morphology, but are still recognizable as such, so that if these are present in the sputum in any case, they should be recognized on a routine smear even after it has been treated with acid.

The finding of tubercle bacilli in the sputum in the second case on two different occasions, plus the gross and microscopic features characteristic of tuberculosis, is sufficient to establish such a diagnosis. It is significant, however, that many more specimens of sputums were examined for acid-fast bacilli in which none were found, and that no one noted the asbestosis bodies in the sputum, although these were not being looked for specifically.

An important additional finding in case 2 was that of asbestosis bodies in the spleen. Although we have diligently searched the literature, mention of such a finding, to our knowledge, never previously has been made. Whether these bodies get there by the blood stream as emboli or whether they are carried there by phagocytic cells, we are not prepared to say and have no evidence on which to base a conclusion. The fact that they are in that organ, irrespective of how they got there, seems to be significant.

It is interesting that neither in case 1 nor in case 2 (despite the marked pulmonary fibrosis and the thickening of the smaller pulmonary arteries) was there any dilatation or hypertrophy of the heart on either side.

#### SUMMARY

Two cases of pulmonary asbestosis are reported. In the first case, the symptoms were relatively unimportant. In the second case, the disease was concomitant with tuberculosis and, with it, was the cause of death. The similarity between some of the features of tuberculosis in childhood and tuberculosis complicated by asbestosis is suggested.

In asbestosis the lung is not the only organ invaded by asbestosis bodies. As we have shown, the spleen and the lymph nodes may harbor large asbestosis bodies.

From the histories there appears to be a further need for study along the lines of prevention of this disease.

# TULAREMIC LEPTOMENINGITIS

REPORT OF A CASE \*

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Francis,<sup>1</sup> in 1928, tabulated twenty-four fatal tularemic infections in man and stated that five of the patients died in the second week of illness in delirium and stupor, the symptoms indicating severe involvement of the meninges (meningitis). The reports in those cases in which necropsies were made say nothing about the brain. Francis and Callender,<sup>2</sup> in their report, had in mind the possibility of lesions of the meninges or of the brain in their reference to the postmortem examination of a Negro in whom tissue reactions resembling those of tularemia were found in the spleen. Because of stupor and other clinical symptoms of meningitis, death was ascribed to tuberculosis. The meninges at the base of the brain were opaque, and microscopically there was a diffuse infiltration by lymphocytes, with considerable thickening and an occasional Langhans' giant cell surrounded by a few epithelioid cells. There was a hyperplasia of the endothelial cells of the capillaries. Francis and Callender stated that the lesion may have been tuberculous, although acid-fast bacilli were not demonstrated. Examinations of the blood and inoculations of guinea-pigs were not made. In the brain of a woman dying on the eighth day after the onset of illness, Palmer and Hansmann<sup>3</sup> noted hyperemia, a minute hemorrhage of the pons, small masses of endothelial cells in the pia-arachnoid, fatty changes of the neurons, glia cells and muscle cells of the blood vessels and a diffuse degeneration of the myelin in the fiber tracts of the pons and in the fibers of the eighth cranial nerve. Replying to an inquiry by Dr. Warren T. Vaughan on involvement of the central nervous system in tularemia, Simpson<sup>4</sup> stated that he did not think that there is an actual

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\* Submitted for publication, July 27, 1931.

\* From the Henry Baird Favill Laboratory of St. Luke's Hospital.

1. Francis, E.: J. A. M. A. **91**:1155, 1928.

2. Francis, E., and Callender, G. R.: Arch. Path. **3**:577, 1927.

3. Palmer, H. D., and Hansmann, G. H.: J. A. M. A. **91**:236, 1928.

4. Simpson, W. M.: J. Lab. & Clin. Med. **15**:311, 1930.

involvement of the central nervous system, but that the nervousness, restlessness and insomnia of the disease are associated with the general debility. Dwijkoff<sup>5</sup> observed regularly a marked hyperemia of the meninges and brain in rodents with tularemia, and sometimes small hemorrhages. In the meninges of one animal, he found a large hemorrhage and a small infiltration.

It is reasonable to expect in a septicemic disease such as tularemia that almost every tissue of the body at some stage of the illness may be infected. While these general references have been made to clinical symptoms of meningitis in tularemia, there seems to be, as yet, no report of reactions of the meninges or of the brain comparable in cell structure to the lesions regularly found in the liver, spleen and lungs of susceptible hosts infected with *Bacterium tularense*. A description of such lesions distributed in the meninges, submeningeal brain tissues, ependyma, subependymal tissues and choroid plexus of a man who died on the sixteenth day after infection is recorded in the following paragraphs.

#### REPORT OF CASE

W. S., white, aged 48, a chef, entered St. Luke's Hospital, Chicago, in the service of Dr. F. M. Miller, Dec. 14, 1930, with a rapid pulse, a high fever, an ulcerated wound of the right middle finger, tenderness of the right axilla and 7,100 leukocytes per cubic millimeter of blood. On December 5, while he was removing pickled rabbit from a jar, the skin on the dorsal surface of the right middle finger opposite the first interphalangeal joint was torn on a bone of the rabbit. The lesion was immediately treated with a solution of iodine. The following day he was indisposed, and two days later he was acutely ill. The wound of the finger was opened, and hot fomentations were applied, but these had little effect on the chills and fever and on a painful swelling in the right axilla. The chills and fever persisted while he was in the hospital, and on the second day after his admission symptoms of meningitis developed. The patient became irrational. The neck was rigid; the pupils were small and sluggish; the Kernig, left Babinski and Oppenheim reflexes were present; the cremasteric reflex was absent, and the knee reflexes were diminished. The spinal fluid was cloudy and under a pressure of 230 mm. of water, it contained 400 cells per cubic millimeter of fluid, of which 16 per cent were polymorphonuclear leukocytes and 84 per cent lymphocytes. The results of globulin tests were positive, and the albumin content was 75 mg. per hundred cubic centimeters. Bacteria were not found in the sediment, stained by Gram's and Ziehl-Neelsen's stains. Cultures of the spinal fluid on the usual mediums were sterile after six days.

Death occurred on December 21, the sixteenth day after infection of the finger, and the postmortem examination of the viscera of the trunk and head, but not of the neck, was made the same day by Dr. Paul J. Breslich and Dr. J. J. Kearns. The essentials of the anatomic diagnosis were: miliary tularemic lesions of the liver, spleen and lungs; marked acute ulcerative and phlegmonous cellulitis of the right middle finger; acute hyperplasia and necrosis of the right axillary, right supraclavicular and biliary lymph nodes; acute tularemic meningitis; edema of the brain;

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5. Dwijkoff, P. P.: *Virchows Arch. f. path. Anat.* **278**:481, 1930.

marked cloudy swelling of the liver, kidneys and myocardium; marked fatty changes of the liver and kidneys; acute hyperplasia of the spleen; acute fibrinous perisplenitis; acute catarrhal tracheitis and bronchitis; bilateral bronchopneumonia; bilateral hydrothorax, and no yellow lipid substance in the suprarenal cortex.

Only the more important observations recorded at autopsy will be mentioned. The body weighed 147 pounds (66.7 Kg.) and was 173 cm. long. There was a slight icterus of the sclerae and of the skin. On the ulnar and dorsal side of the first interphalangeal joint of the right middle finger was an ulcer 15 mm. in diameter. The tissues of the finger were edematous, and the skin was wrinkled from wet dressings. The subcutaneous tissues of the front of the chest were edematous; the abdomen contained 85 Gm. of a clear, yellow fluid. The lymph nodes of the right axilla were as large as 30 by 15 by 10 mm., and on cut surfaces were dark red and about 50 per cent mottled with necrotic regions, some liquefied. The spleen weighed 400 Gm. Many yellow and gray regions, from 1 to 2 mm. in diameter, and petechial hemorrhages were visible through the capsule. The capsule of the diaphragmatic surface was roughened by organizing fibrin. On cut surfaces, the spleen was soft, dark red and mottled with many discrete yellowish-white regions, 1 mm. in diameter. The liver weighed 1,950 Gm. The capsule was smooth. The lobular markings were faint, and disseminated in the brown tissues without regular order were regions varying in color from grayish white to yellow, and measuring 1 mm. or less in diameter. There was marked cloudy swelling of the liver. The biliary lymph nodes were as large as 25 by 15 by 7 mm., and opaque, white lymphoid tissue was mottled with yellow regions of necrosis. The right lung weighed 850 Gm. The pleura was smooth, except dorsally where there were a few torn fibrous adhesions. The lobular margins were pigmented with carbon, and the parenchyma contained many shotty nodules of white tissue from 2 to 5 mm. in diameter. These, on cut surfaces, stood out from the surrounding dark-red lung substance as firm, white, granular nodules resembling miliary tubercles. In addition, there were red, granular bronchopneumonic consolidations of the lung about 1 cm. in diameter. The lining of the bronchi was hyperemic. The left lung weighed 800 Gm. and in all essentials was like the right lung. It had many of the widely disseminated nodules of white tissue. There were no active tuberculous lesions or other significant changes in the viscera except those of tularemia, a conclusion supported by the routine histologic examinations of these tissues, which showed focal tularemic lesions in the lungs, liver, spleen and lymph nodes.

The histologic structure of the lesions in tularemia has been described.<sup>6</sup> The lesions in the liver and spleen of this patient were in the stage in which necrosis and nuclear fragmentation of the exudate cells are predominant. The lesions of the lungs had, in addition, reactive changes along their margins.

Serum removed from the patient on the fourteenth day of the illness, two days before death, was sent to the Hygienic Laboratory of the United States Public Health Service and was reported to agglutinate *Bacterium tularense* in dilutions not greater than 1:40. Dr. Francis, in a comment on this report, stated that "while the titre is low, it is in keeping with that of serum collected on the fourteenth day of tularemia, and especially in a person who has failed to form antibodies of any sort as indicated by his death on the sixteenth day of illness."

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6. Francis and Callender (footnote 2). Goodpasture, E. W., and House, J.: *Am. J. Path.* 4:213, 1928. Simpson, W. M.: *Arch. Path.* 6:553, 1928.

Two guinea-pigs (1 and 2) were inoculated on December 22 with crushed spleen. Both animals died on December 27, and the spleen, liver and lung had focal lesions characteristic of tularemia. Guinea-pig 3, inoculated with splenic pulp of guinea pig 1, died on Jan. 19, 1931, and at the site of the inoculation was granulation tissue microscopically identical with that found in the lesions of guinea-pig 1. Guinea-pig 4 was inoculated on January 27 with crushed spleen obtained from the body of the patient and stored in the icebox for one month. This animal remained healthy. *B. tularensis* was not isolated in cultures of the human and animal tissues. Human lung, liver and spleen and also liver and spleen from guinea-pig 1 were sent to Dr. Francis for examination, who stated that the guinea-pig tissues had the usual lesions found with tularemia. The human liver and spleen had similar focal lesions, and the lungs an exudative pneumonitis and in one place a focal tularemic lesion.

These conditions in the viscera of the trunk are given with considerable detail in order to present adequately the evidence favoring the tularemic nature of the disease. They are important, of course, but are incidental to the chief portions of this report, namely, those that follow, describing the lesions of the meninges and brain.

There were no changes in the blood sinuses of the dura or in the right and left middle ears or in the sphenoid, ethmoid and frontal cranial sinuses. A large amount of slightly turbid spinal fluid escaped when the brain was removed. The hyperemic leptomeninges were slightly opaque with a diffuse exudate and contained many miliary opacities from 1 to 2 mm. in diameter and less. After hardening in 8 per cent formaldehyde, the brain weighed 1,270 Gm. The hemispheres were asymmetric: the right was 17 cm. long, 8 cm. wide and 7 cm. thick in the temporal region; the left, 17 cm. long, 8.5 cm. wide and 6.5 cm. thick. The convolutions were flattened, the sulci shallow and narrow. The brain substance was uniformly firm. There was a milky opacity of the leptomeninges covering the superior and lateral surfaces of the hemispheres, especially along the sulci and in the fissures of Sylvius. Focally, they were raised slightly from the underlying cortex and contained discrete white nodules from 1 to 2 mm. in diameter, especially along the course of the blood vessels in the precentral and postcentral gyri. The blood vessels of the leptomeninges were distended with blood. The basilar and vertebral arteries were absent. The circle of Willis was intact and unchanged. The basal leptomeninges had a milky opacity and were raised from the convolutions and thickened. The olfactory bulbs, optic chiasma and third, fourth, fifth and sixth cranial nerves, the anterior and posterior perforating substance with their blood vessels, the infundibulum and the mammillary bodies were unchanged. The cerebellar peduncles were symmetrical, measuring 2.2 by 1.7 cm. The red nuclei and the substantia nigra were unchanged. The pineal body was 8 by 5 by 5.5 mm., the tissue firm and granulated.

With a binocular magnification of 10.5 diameters, the leptomeninges had disseminated milky opacities, especially distinct over the blood vessels in the sulci; over the blood vessels, they were edematous and lifted away from the cortex. Also along the blood vessels and adherent to or forming a part of the meninges were small multiform plaques, 1 mm. in diameter, like tubercles, but less opaque, and milky white. The opacity of these plaques was so great that the underlying blood vessels were hidden or had a yellow tinge. The opacity of the meninges and the opaque plaques extended over the base of the brain, but not to such a marked extent.

The cerebrum was sectioned frontally in parallel planes about 1 cm. apart. There was a slight increase of the stippling. The gray cortex had an average thickness of from 2.5 to 5 mm. and was sharply demarcated from the white substance. There were no hemorrhages or soft regions in the tissues of the basal ganglions. The lateral ventricles were symmetrical and small, and at the level of the anterior commissure they were 2.8 cm. wide. The third ventricle was unchanged. The ependymal lining of the third and especially of the lateral ven-

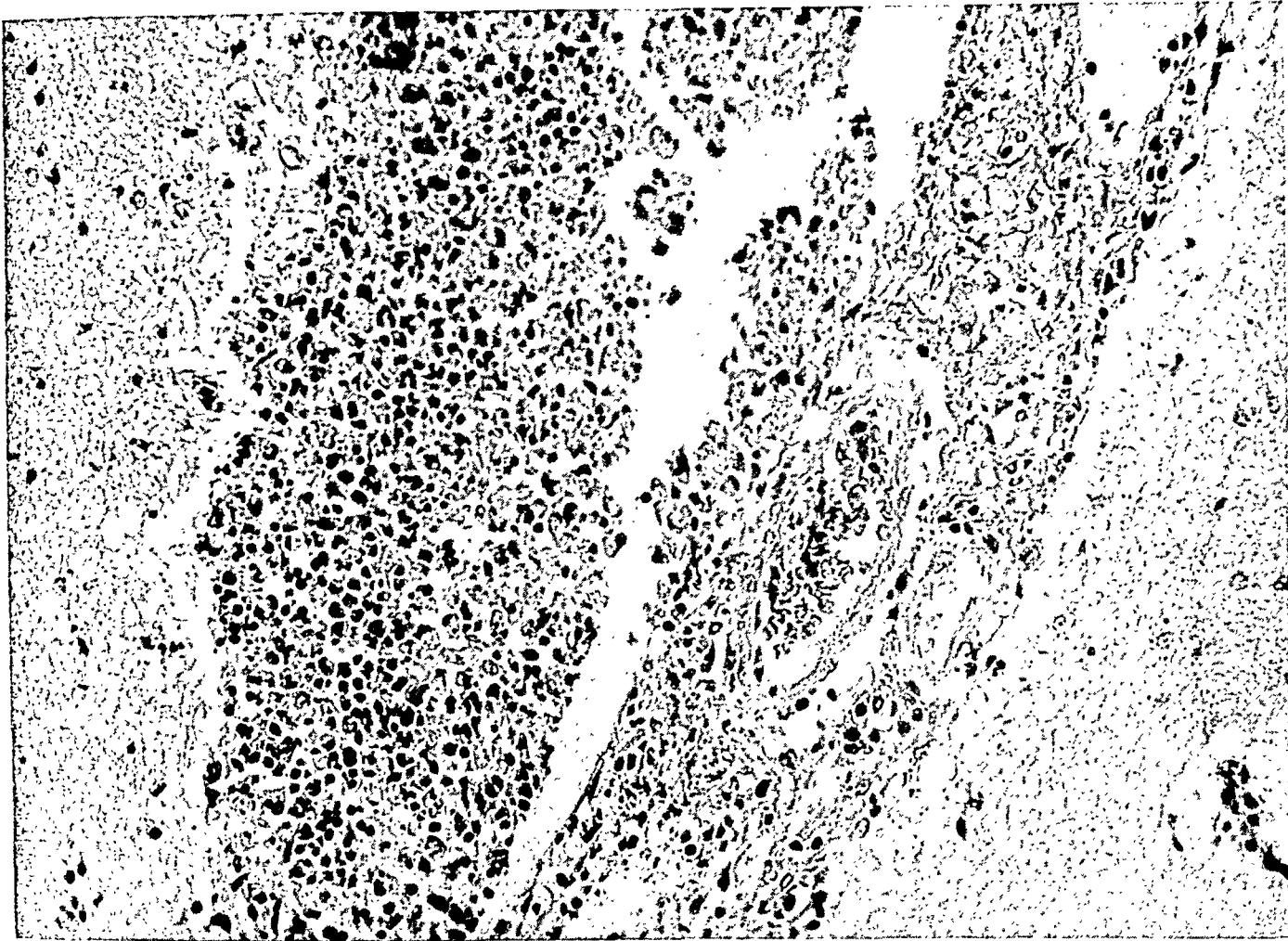


Fig. 1.—A focal lesion of the meninges deep in a sulcus, with involvement of the brain;  $\times 252$ .

tricle was roughened by slightly raised, white nodules less than 1 mm. in diameter. The choroid plexus varied in color from gray to brown and was unchanged. The cerebellar hemispheres were symmetrical and measured 5 by 6 by 5 cm. The pons was 3 by 3.5 by 3 cm. and had 3.8 cm. of the brain stem attached. The pons, brain stem and cerebellum showed no noteworthy changes.

Twenty-eight blocks of tissue were taken for histologic examination from various places of the cortex, the white substance, the principle ganglion centers and tracts, the cerebellum and the choroid plexus of the lateral and third ventricles. They were embedded in paraffin, and most of the sections were stained with hema-



toxylin and eosin, a few with phosphotungstic acid-hematoxylin and otherwise. The nodular opacities in the leptomeninges were masses of exudate cells, chiefly polymorphonuclear leukocytes, large mononuclear cells, plasma cells and cells with a large, faintly stained nucleus and a granular, fairly abundant cytoplasm (fig. 1). These cells were in dense aggregates from 0.5 to 1 mm. in diameter, and were held in meshes of fibrin threads. Most of these focal lesions were in the leptomeninges, but some encroached on the adjacent brain substance and penetrated to a depth

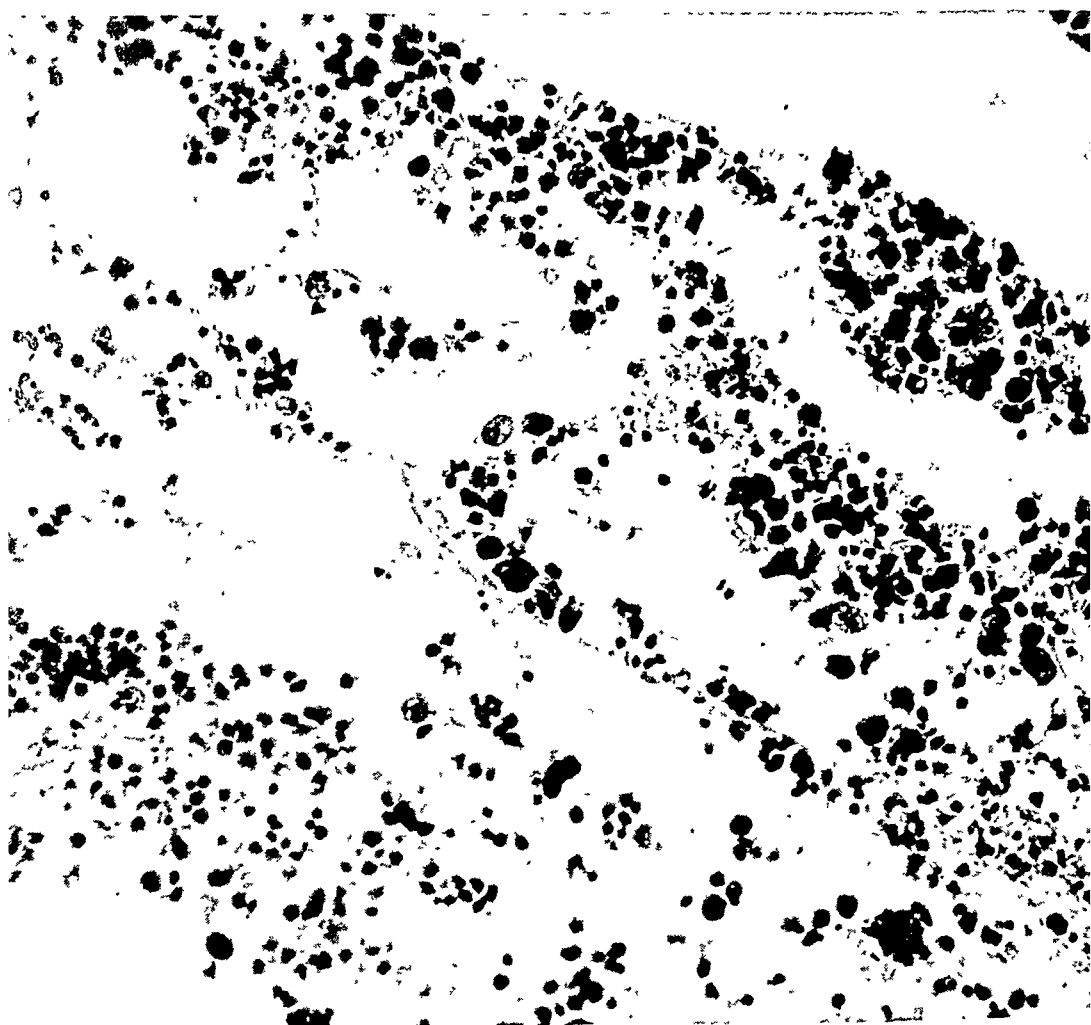


Fig. 2.—A diffuse exudative reaction in the meninges;  $\times 252$ .

of from 0.2 to 0.5 mm. In such places the brain tissues were necrotic and infiltrated by dense masses of polymorphonuclear leukocytes and large mononuclear exudate cells. The nuclei of many of the exudate cells were pyknotic and fragmented. A study of all these sections demonstrated that the lesions were distributed chiefly, although not invariably, along the blood vessels. In addition to these focal lesions there were diffuse exudative cellular reactions of certain portions of the leptomeninges (fig. 2). Focal lesions were present also on the ependymal lining of the lateral and third ventricle and in the epithelium of the choroid plexus. They were not found isolated in the white substance of the cerebrum or in that of the cerebellum. The leptomeninges of the spinal cord of the first segment had lesions like

those described in the pia-arachnoid of the brain. Acid-fast bacilli were not found in lesions of the liver, spleen and leptomeninges (from three separate places) stained according to a technic that in control tuberculous tissue demonstrated acid-fast organisms.

#### SUMMARY

Tularemic lesions of the leptomeninges, contiguous brain tissues, ependyma, subependymal tissues and choroid plexus of a man dying on the sixteenth day following laceration of a finger by contact with a rabbit bone had the histologic structure of similar lesions in the liver, spleen and lungs, and corresponded structurally with those described in the visceral tissues of susceptible hosts dying with *B. tularensis* infection.

The lesions grossly resembled miliary tubercles, although their distribution was chiefly over the vertex and sides rather than along the base of the brain, and consisted of exudates of monocytes and polymorphonuclear leukocytes with varying degrees of necrosis. Where the meningeal lesions encroached on the brain, the tissues of the latter were necrotic and infiltrated by exudate cells.

In addition to the focal lesions of the meninges there was a diffuse, acute exudative meningitis. Widely disseminated lesions of the brain were not demonstrated.

Although, generally speaking, the lesions of tularemia mimic those of tuberculosis, it is unlikely that the reactions found in the meninges of this patient were tuberculous, because (1) they repeated in minute detail the structure of lesions found in the spleen, and suspensions of these crushed tissue killed guinea-pigs in five days and in them produced visceral lesions characteristic of tularemia; (2) the search for acid-fast bacilli in the lesions of the meninges, liver and spleen was not successful, and (3) lesions of a frankly tuberculous character were not found by thorough gross and histologic examinations of the viscera.<sup>7</sup>

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7. Since the completion of this manuscript, Dr. F. W. Hartman of the Henry Ford Hospital, Detroit, has sent us a copy of his report concerning tularemic encephalitis. His forthcoming article describes lesions in the brain substance, but none in the meninges. In this respect, his observations differ from those in our report.

# EXPERIMENTAL ARTERITIS AND ARTERIOSCLEROSIS ASSOCIATED WITH STREPTOCOCCAL INOCULATIONS\*

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In 1920, one of us observed early aortic arteriosclerosis in a young girl, 14 years of age, with gangrenous stomatitis and fatal septicemia. Cultures from the mouth before death and of the blood at autopsy yielded a mixture of *Streptococcus hemolyticus* and *Staphylococcus aureus*. Autopsy revealed septic infarcts of the heart and lungs, disseminated acute myocarditis, thrombi in the right auricle and ventricle and fresh, patchy fatty degeneration of the aorta and larger arteries, with beginning arteriosclerosis. Microscopic examination disclosed intimal thickening, consisting of atheromatous ulcers and arteriosclerosis, involving the entire circumference of the aorta. The media, and in some places the intima, contained blue-staining streaks of homogenous material, which separated the smooth muscle layers. Sudan III-hematoxylin stains demonstrated a lipoid infiltration of the intima. The typical histologic picture of beginning arteriosclerosis was present, and the question then arose whether it was due to the associated infection.

Some time later, we began to culture the coronary arteries of arteriosclerotic patients, particularly in fatal cases of coronary thrombosis. In three instances, *Streptococcus viridans* was found in the coronary arteries, although cultures of the blood were negative. From one heart we cultured a sclerotic coronary artery, and from two others thrombi in sclerotic coronary vessels. Subsequent intravenous inoculations of rabbits with the recovered streptococci demonstrated the ability of the latter to persist in the blood stream for from three to five days. We considered that these organisms were probably pathogenic and decided to inoculate a series of rabbits in order to determine their effect on the heart and blood vessels.

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## INOCULATION OF RABBITS

*Series 1.*—Litter 1, of seven rabbits, each of which was inoculated intravenously with a massive dose of streptococci from one of the cases, was observed for about a week and then killed. Although blood cultures showed that the organisms could be recovered for from three to five days after inoculation, autopsy disclosed no significant cardiovascular changes, except a slight lymphoid infiltration of the interstitial tissue of the myocardium. As a single massive inoculation failed to produce changes, we decided to make repeated inoculations over a prolonged period of time.

*Series 2.*—Litter 2 consisted of seven young rabbits. Five received seven inoculations each with *Streptococcus fecalis* from a coronary artery in a period of five and one-half months. Rabbit 5, in addition to its inoculations, had thirty-three



Fig. 1.—Arteriosclerosis of the aorta of rabbit 5, series 2, inoculated with *Streptococcus viridans* from a sclerotic coronary artery.

cholesterol feedings of 0.5 Gm. each. Rabbit 6 had only cholesterol, thirty-three feedings. Rabbit 7 was saved as a control. All seven were killed at the end of six months.

Only rabbit 5 showed any gross changes in the aorta (fig. 1). The ascending aorta contained thickened, yellowish, ulcer-like plaques, in which the intima was considerably raised. There was also a more diffuse irregular thickening of the whole ascending aorta and to a lesser extent of the descending aorta. Microscopic examination disclosed decided thickening and irregularity of the intima with considerable fibrous increase and scarring and with obscuring of the lamina elastica interna (fig. 2). Fibroblasts were scattered throughout the intima, and a few vacuoles were present. The intima also contained elongated streaks of blue-staining material. Sudan III-hematoxylin stains disclosed a diffuse infiltration by small globules of lipin throughout the intima. The arteriosclerotic process,

which involved the intima and to some extent the media, had nearly passed the atheromatous phase and had undergone fibrosis with considerable scarring.

Litter 3, consisting of two young rabbits, without controls, received three inoculations with the same strain of *S. fecalis*, in a period of two months. At autopsy, one of these showed small atherosclerotic plaques in the ascending aorta; the other did not.

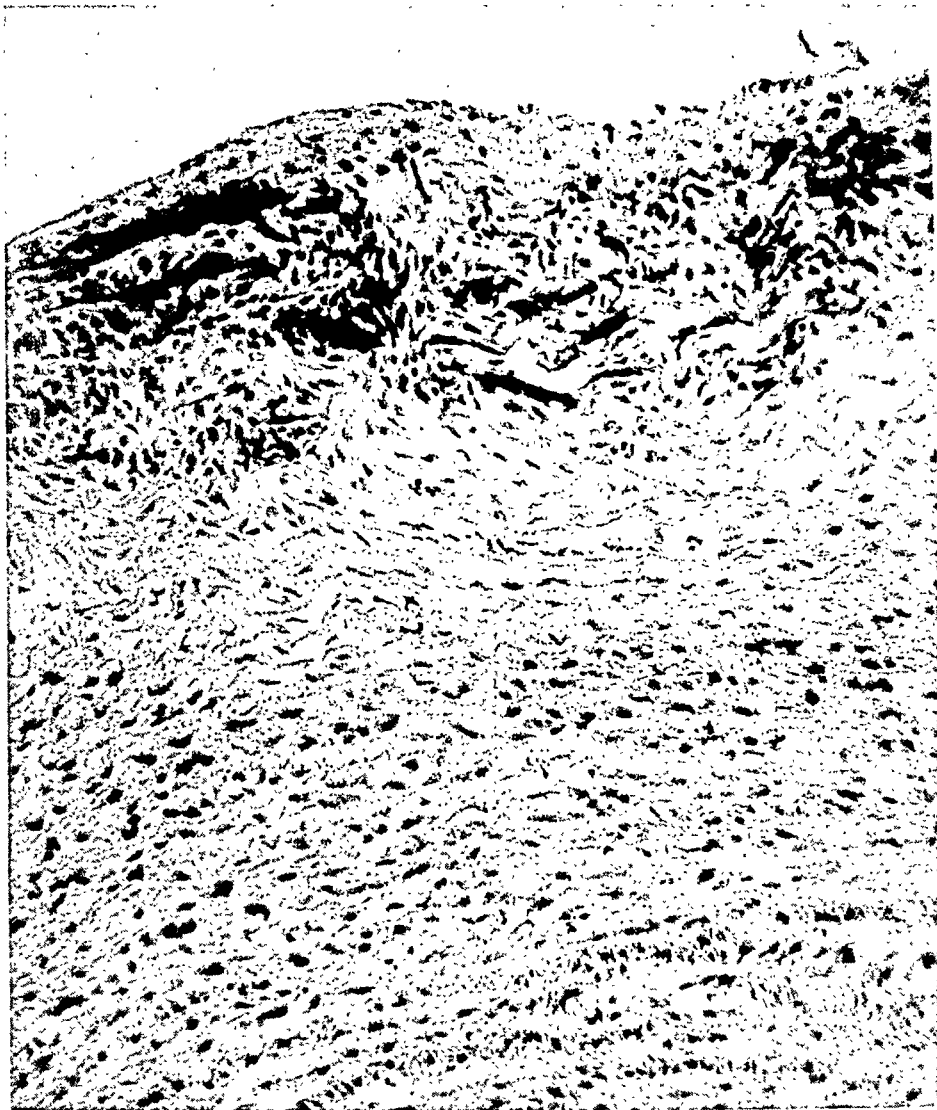


Fig. 2.—Photomicrograph of the aorta of rabbit 5, series 2, showing fibrous thickening and scarring of the intima and cellular infiltration of the adventitia and media.

Litter 4 consisted of six rabbits, two of which were kept as controls. Of the others, three received two inoculations each with the streptococcus, and another received four inoculations. The inoculated animals were also given a subcutaneous implantation of 2 cm. of aseptic rabbit aorta, in the hope of inducing absorption of enough aortic substance to influence the electivity of the injected organisms for aortic tissue. The organisms used had been repeatedly cultivated over a period of many days in dextrose infusion broth containing a portion of sterile

rabbit aorta. One rabbit died one month after inoculation, with atherosclerotic plaques in the aorta. The other five rabbits, including the controls, lived from one to three months and showed no gross lesions.

Litter 5 consisted of seven rabbits. Two were kept as controls, and the others received two inoculations with the streptococcus. Autopsies on all of them, four months later, showed no gross changes.

*Series 3, Inoculated with Streptococcus Viridans from a Coronary Thrombus.*—A thrombus in the coronary artery of a man, 66 years of age, who died of generalized arteriosclerosis with coronary thrombosis was cultured two hours after the patient's death. At the same time, the blood in the heart was cultured and found to be sterile. The thrombus was cultured in dextrose infusion broth enriched with sterile human blood, and on blood agar plates. The plates yielded many colonies of a green-producing nonhemolytic streptococcus, and the broth gave a good growth of long chain, lanceolate diplostreptococci. This was identified by growth in lactose, mannite and salicin as nonhemolytic streptococcus type III (Holman).

TABLE 1.—*Observations in Litter 6, Inoculated with Strain of Streptococci from Coronary Thrombus*

Rab- bit	Procedure	Duration, Mo.	Fate	Lesions		Pathologic Changes
				Aortic Le- sions	of Smaller Arteries	
1	27 inoculations with streptococci	8	Died	0	0	Pericarditis
2	27 inoculations with streptococci	10	Killed	0	+	Sclerosis of arteries of lung
3	27 inoculations with streptococci; 127 cholesterol feedings	9	Killed	+	+	Large, flat, whitish atheromatous plaques, microscopically arteriosclerotic
4	27 inoculations with streptococci; 127 cholesterol feedings	8	Killed	0	+	Intimal thickening and sclerosis of small arteries in lung and liver; endarteritis obliterans
5	131 cholesterol feedings.....	9	Killed	0	0	None
6	Control.....	9	Died	0	0	Pneumonia

All rabbits used in our experiments were young, healthy animals, weighing from 2 to 3 pounds (907 to 1,360 Gm.) each, and great care was given to their feeding and hygiene. The diet consisted of fresh vegetables with a regular ration of oatmeal, and whenever cholesterol was fed, it was with oatmeal. On several occasions, inoculations were discontinued for a period of time and repeated blood cultures made to determine the duration of survival of the organisms in each animal's blood stream. This period was found to vary from one to two weeks. In the course of repeated inoculations in the aural vein, abscesses were eventually produced in the tissue of the ear, and these may have accounted for the persistence of the organisms in the blood stream.

Litter 6 consisted of six rabbits. Rabbits 1 and 2 were each inoculated intravenously twenty-seven times in a period of eight months with the streptococcus grown in dextrose infusion broth. Rabbits 3 and 4 were similarly inoculated but, in addition, each received 127 cholesterol feedings of 0.5 Gm. Rabbit 5 had the cholesterol feedings only, and no. 6 was saved as a control. The findings among the various rabbits in this litter are indicated in table 1.

Rabbit 3, which survived nine months of inoculations and cholesterol feedings, presented large, flat, whitish atheromatous plaques of the aorta (fig. 3). Micro-

scopic examination of the aorta showed a thickened intima with lipoid infiltration (fig. 4). Very little cellular infiltration was present. The smaller arteries of the lung, kidney and brain showed irregular thickening with moderate fibrous increase.

Rabbit 4 showed no gross lesions. Microscopic examination disclosed irregular thickening of the walls of the smaller arteries of the lung, with fibrous increase and, in some cases, almost complete closure of the lumen. The proliferation was chiefly intimal, with considerable hyaline change. In some of the arterioles, proliferation of the intimal cells nearly filled the lumen, and in other cases the occlusion was completed by a hyaline substance. In the larger arteries, cellular proliferation was evident and a crescentic thickening of the intima sometimes encroached appreciably on the lumen, almost occluding it. In the lung, three arteries, one about 2 mm., and the others 1 mm., in diameter, were infiltrated by



Fig. 3.—Extensive early arteriosclerosis in the aorta of rabbit 3, litter 6, inoculated with *Streptococcus viridans* from a coronary thrombus.

mononuclears and polymorphonuclears. This was especially true of the intima, which was thickened, hyalinized and densely infiltrated by polymorphonuclears, and which blended off into a laminated red thrombus that occluded the dilated lumen of the artery (fig. 5). Portions of the thrombus contained dense accumulations of pyknotic nuclei. In the kidney, there was thickening of the intima of the arteries with endothelial cell proliferation and lipoid infiltration. The walls were infiltrated by clusters of lymphoid cells and polymorphonuclears. The arterial findings included endarteritis obliterans, arteriosclerosis, mainly intimal, and endarteritis of branches of the pulmonary artery, with occluding thrombosis. Rabbit 2 also had sclerosis of the smaller arteries of the lung.

Litter 7 consisted of six rabbits. Rabbits 1 and 2 were inoculated twenty-seven times each in eight months with the streptococcus. Rabbits 3 and 4, in

addition to these inoculations received cholesterol feedings of 0.5 Gm. each. Rabbit 5 had only cholesterol feedings, and rabbit 6 was saved as a control (table 2).

Rabbit 3 survived ten months and had small, whitish sclerotic plaques in the ascending aorta. In rabbit 4, one arterial branch in the lung was nearly occluded by an old thrombus with fibrosis. A large area of lung in the immediate vicinity of this vessel was necrotic. Several other arteries had thickened walls with intimal proliferation (fig. 6).

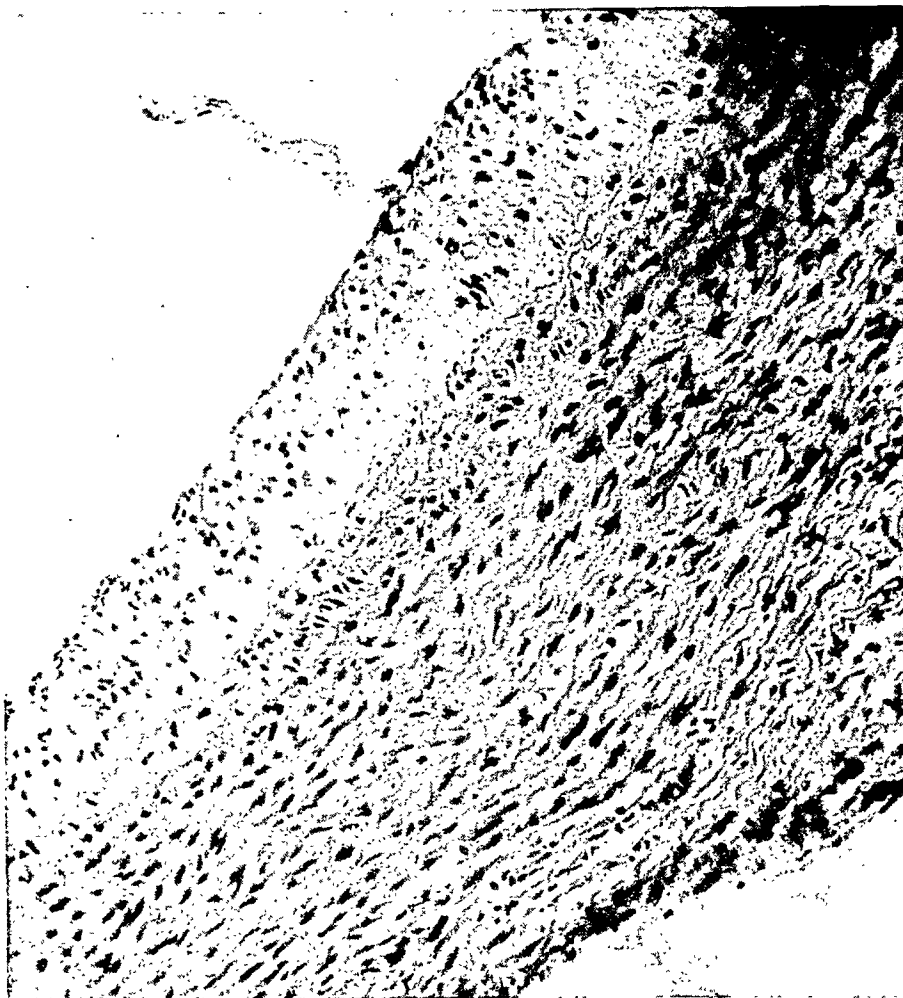


Fig. 4.—Photomicrograph of the aorta of rabbit 3, litter 6, showing moderately early arteriosclerosis with thickening of the intima characterized by loose tissue.

Litter 8 consisted of five rabbits. Each of these was inoculated intravenously seven times with the streptococcus over a period of about seven months (table 3).

Rabbit 2 showed indurated ulcers of the aorta. Microscopic study revealed early arteriosclerosis of the aorta. One small artery in the lung had a thick wall, with roughening of the intima on one side and an attached thrombus of fibrin and polymorphonuclears filling one third of the lumen. Rabbit 1 exhibited slight intimal thickening of the smaller arteries of the lung. Rabbit 3 showed intimal thickening and hyaline change in a medium-sized branch of a coronary artery, with dense, patchy lymphoid infiltration of the media. The arterial findings



revealed chronic indurative arteritis and endarteritis with hyaline changes and irregular scarring of the walls. The control rabbit, no. 5, died of pneumonia after two months' observation, and microscopic examination disclosed thickening of the intima and media in the smaller arteries of the lung, a slight thickening of a branch of a coronary artery and acute glomerulotubular nephritis. It was possible that the arterial changes and nephritis in this rabbit resulted from the pneumonia.

TABLE 2.—*Observations in Litter 7, Inoculated with Strain of Streptococci From Coronary Thrombus*

Rabbit	Procedure	Duration, Mo.	Fate	Lesions		Pathologic Changes
				Aortic Lesions	of Smaller Arteries	
1	27 inoculations with streptococci	8	Died	0	0	Bronchopneumonia
2	27 inoculations with streptococci	10	Killed	0	0	None
3	27 inoculations with streptococci; 131 cholesterol feedings	10	Killed	+	0	Small sclerotic plaques of aorta
4	23 inoculations with streptococci; 62 cholesterol feedings	3	Died	0	+	Arteriosclerosis and thrombosis of medium-sized arteries of lung; infarct of lung; septicemia
5	131 cholesterol feedings.....	10	Killed	0	0	None
6	Control.....	10	Killed	0	0	Chronic diffuse nephritis

TABLE 3.—*Observations in Litter 8, Inoculated with Strain of Streptococci from Coronary Thrombus, Grown in Aorta Medium, and Given Subcutaneous Implantation of Aorta Tissue*

Rabbit	Procedure	Duration, Mo.	Fate	Lesions		Pathologic Changes
				Aortic Lesions	of Smaller Arteries	
1	Aorta implanted; 7 inoculations with streptococci	7	Killed	0	±	Round cell accumulations in heart
2	Aorta implanted; 7 inoculations with streptococci; 50 cholesterol feedings	7	Killed	+	+	Atheromatous ulcers of aorta; sclerosis of smaller arteries with thrombosis of one in lung
3	7 inoculations with streptococci; 50 cholesterol feedings	7	Killed	0	+	Sclerosis of coronary artery and of arteries in lung
4	50 cholesterol feedings.....	7	Killed	0	±	Lipoid infiltration of intima of small coronary branch
5	Control.....	2	Died	0	+	Nephritis; sclerosis of small arteries

*Series 4, Inoculated with Hemolytic Streptococci from Sinuses.*—Two cultures of hemolytic streptococci from the sphenoid sinuses of a patient with arthritis and chronic sinusitis were obtained for study and both typed out according to Holman's classification as *Streptococcus anginosus*. In an effort to determine whether sinus infection might cause arterial changes, a series of inoculations was started with these organisms.

Litter 9 consisted of seven rabbits of which rabbits 1 to 4 were inoculated ten times each with a sinus streptococcus over a period of about six months. Rabbit 5 received inoculations and cholesterol feedings. Rabbits 6 and 7 were saved as controls (table 4).

These inoculations with hemolytic streptococci were attended by arterial changes. Rabbits 1, 2 and 5 exhibited gross lesions of the aorta. No. 1 had small, raised atheromatous plaques. Microscopic examination of the lung disclosed intimal proliferation with deposition of fibrin in some of the smaller arteries (fig. 7). In places, arteritis was more active. Collections of fibrin and leukocytes were attached to the roughened intima and invaded the wall of the blood vessel. Pneumonia was not present.

The ascending aorta of rabbit 2 showed several whitish plaques. Microscopically there was patchy intimal thickening with dense infiltration by fibroblasts. Small

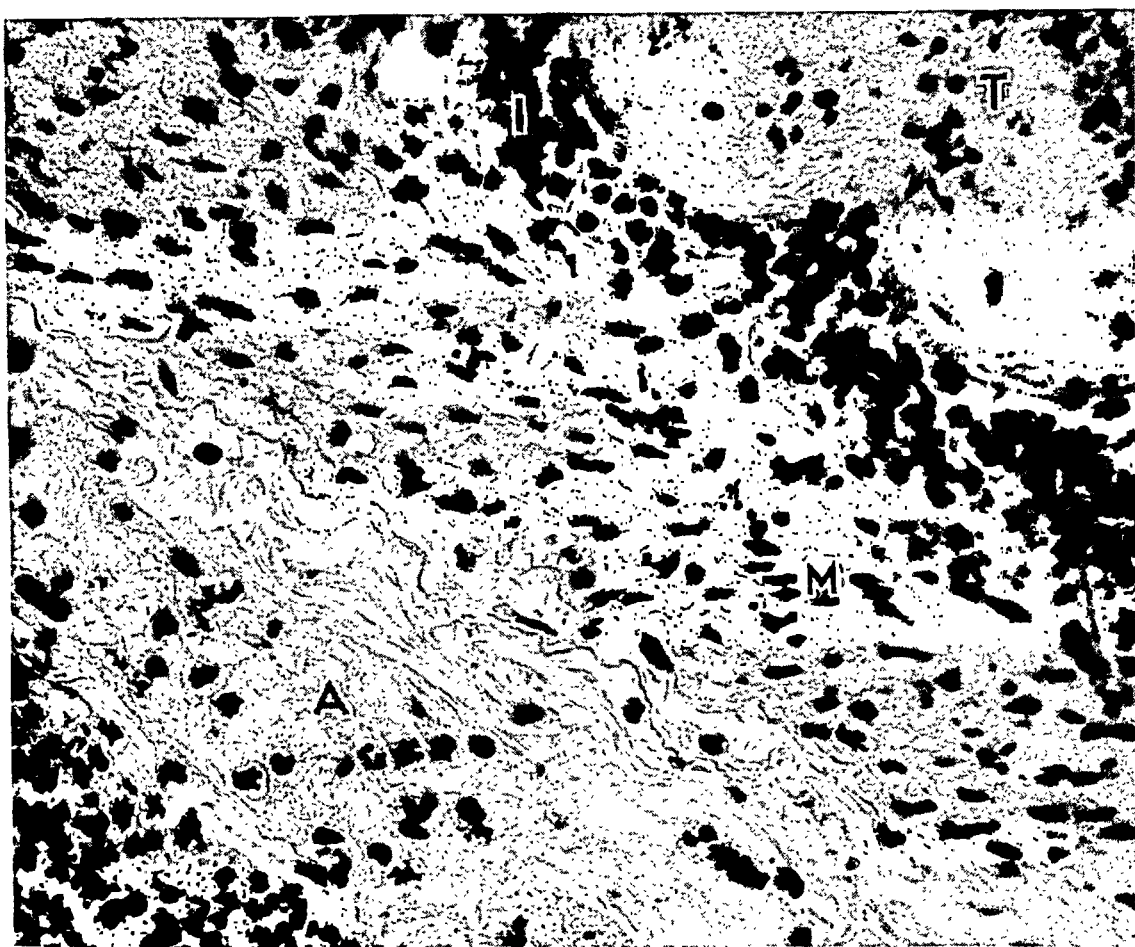


Fig. 5.—Photomicrograph of the wall of an artery of the lung in rabbit 4, litter 6, inoculated with *Streptococcus viridans* from a coronary thrombus and showing acute arteritis with thrombus formation: *I*, the intima, infiltrated with leukocytes; *M*, the media, showing fibrosis and hyaline change; *A*, the adventitia, densely infiltrated with polymorphonuclear leukocytes; *T*, the thrombus in the lumen.

areas of scarring were noted in the intima and media. In the myocardium, round cell infiltrations occurred. In the kidney, the arteries had thickened walls, often with complete obliteration of the lumina owing to proliferation of faintly stained cells that appeared to be endothelial. Many hyalinized arterioles were found. In all the arteries there was intimal thickening, usually with hyalinization of the

entire wall. In one arteriole, the wall contained a compact infiltration of mononuclear cells. In the lung, the arteries had thickened walls, with scarring of the media and roughening of the intima, on which fibrin was deposited.

Rabbit 5 lived four months, and at autopsy had large, whitish plaques in the aorta, confirmed by microscopic examination. In rabbit 3, some pulmonary arteries showed crescentic thickening of the intima. In rabbit 4, also, there was intimal thickening of the arteries of the lung.

Litter 10 consisted of six rabbits. The first four were inoculated with a hemolytic streptococcus, rabbits 5 and 6 being saved as controls. Only one

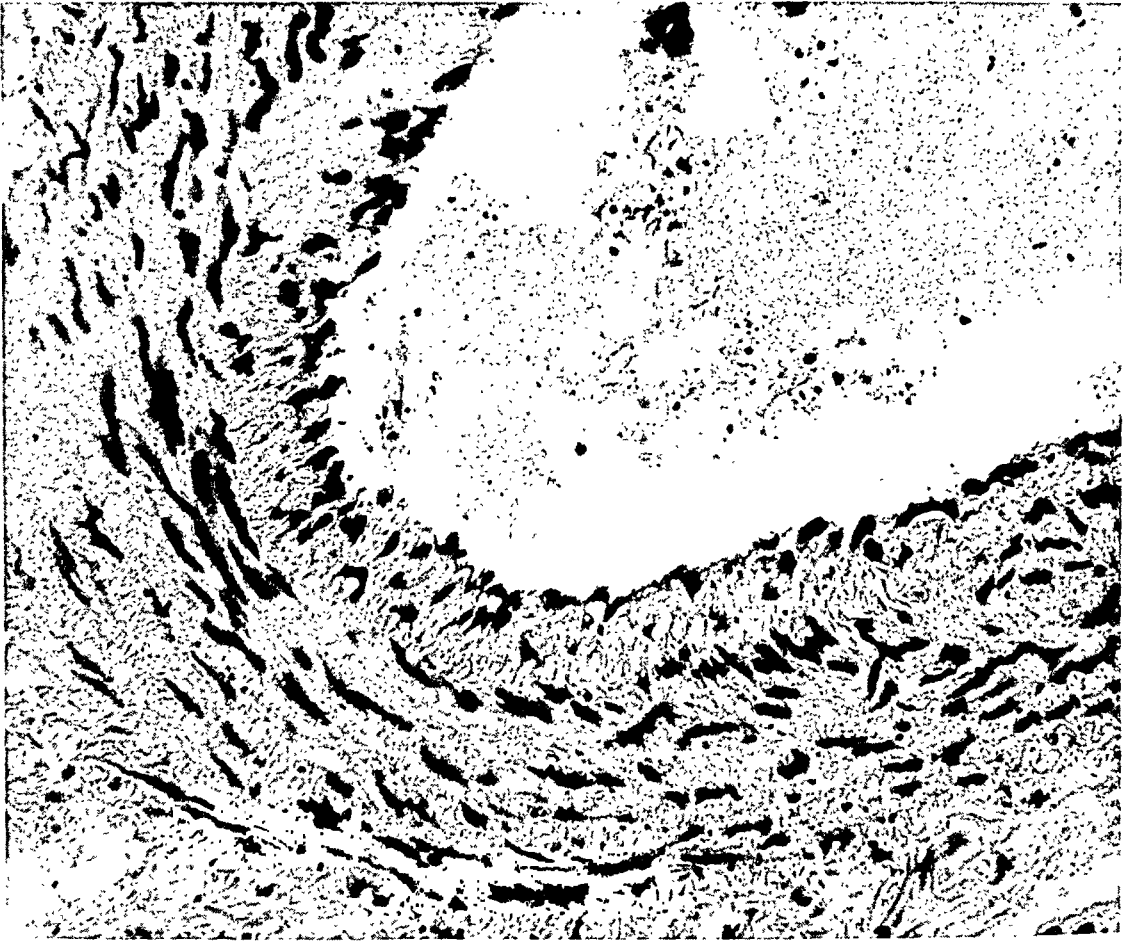


Fig. 6.—Photomicrograph of an artery of rabbit 4, litter 7, showing moderate thickening of the intima in early arteriosclerosis following inoculations with *Streptococcus viridans* from a coronary thrombus.

inoculated rabbit of this litter lived over one month. The others, and even one of the two controls, succumbed to pneumonia.

Rabbit 3, at autopsy, showed small, yellowish, indurated atheromatous ulcers of the ascending aorta. Microscopic examination of the lung disclosed irregular intimal thickening in medium-sized arteries. In one artery, the intima was greatly thickened and densely infiltrated by mononuclear cells having irregular or spindle-shaped nuclei. The underlying media had undergone hyalinization and lipoid infiltration. In the myocardium there were focal accumulations of lymphocytes.

Microscopic examination of rabbit 1 showed multiple myocardial abscesses, pneumonia and acute diffuse nephritis, with a hyaline change in the arteries of the kidney. Rabbit 2 died of pneumonia, and microscopic examination showed acute arteritis in the lung.

#### INOCULATION OF MONKEYS

*Series 5.*—Twelve monkeys (*Macacus rhesus*) were given a diet of vegetables, bread, milk, grains and nuts. One control was killed at the beginning of the

TABLE 4.—*Observations in Litter 9, Inoculated with Strain of Streptococcus Anginosus from Sphenoid Sinus*

Rab- bit	Procedure	Duration, Mo.	Fate	Lesions		Pathologic Changes
				Aortic Le- sions	of Smaller Arteries	
1	10 inoculations with streptococci	6	Killed	+	+	Purulent arteritis in lung; atheromas of aorta
2	10 inoculations with streptococci	6	Died	+	+	Pneumonia and nephritis; arteritis in lung
3	10 inoculations with streptococci	9	Killed	—	+	Sclerosis of arteries of lung
4	10 inoculations with streptococci	9	Killed	0	0	None
5	10 inoculations with streptococci; 101 chlesterol feedings	4	Died	+	0	Atheroma of aorta
6	Control.....	9	Killed	0	0	None
7	Control.....	9	Killed	0	0	Interstitial nephritis

TABLE 5.—*Observations in Litter 10, Inoculated with Strain of Streptococcus Anginosus from Sphenoid Sinus*

Rab- bit	Procedure	Duration, Mo.	Fate	Lesions		Pathologic Changes
				Aortic Le- sions	of Smaller Arteries	
1	5 inoculations with streptococci	1	Died	0	+	Multiple abscesses of myo- cardium; purulent arter- itis in lung
2	5 inoculations with streptococci	1	Died	0	+	Pneumonia; acute arteritis in lung
3	6 inoculations with streptococci	7	Killed	+	+	Small, indurated atherom- atous ulcers of aorta; sclerosis of arteries of lung
4	5 inoculations with streptococci	½	Died	0	0	Pneumonia
5	Control.....	½	Died	0	0	Pneumonia
6	Control.....	7	Killed	0	0	None

experiment for histologic preservation of the tissues and at the end of the experiment comparison was made with the two controls on which autopsies were made one year later. Eight monkeys were inoculated twenty-four times with streptococci (twice washed saline suspensions containing two billion bacteria per cubic centimeter) in a period of six months. Each animal was inoculated just below Poupart's ligament in the immediate vicinity of the right femoral artery. The initial dose of 1 cc. was gradually increased in five months to a final dose of 16 cc. At the end of six months the bacterial inoculations were discontinued, and the animals were permitted to live unmolested for another six months; then they were all killed and examined grossly and microscopically. Two controls were allowed to live with the inoculated animals. Monkeys 1 to 4 had been inoculated with a

succession of different strains from various cultures of streptococci, so that reinfection with a new strain had occurred from time to time. In monkeys 1 and 2, hemolytic *S. anginosus*, and in monkeys 3 and 4 nonhemolytic *S. mitis*, had been used. Monkeys 5 to 8 had received pure cultures; in monkeys 5 and 6, we used a strain of hemolytic *S. anginosus* from a patient with mastoiditis and extensive sinus thrombosis, and in monkeys 7 and 8 a strain of nonhemolytic *S. mitis* from the antrum of a patient with chronic sinusitis. In general, the animals remained in good health during the entire year of observation, but monkeys 7 and 8 acquired large inguinal abscesses, which healed spontaneously, and monkey 6

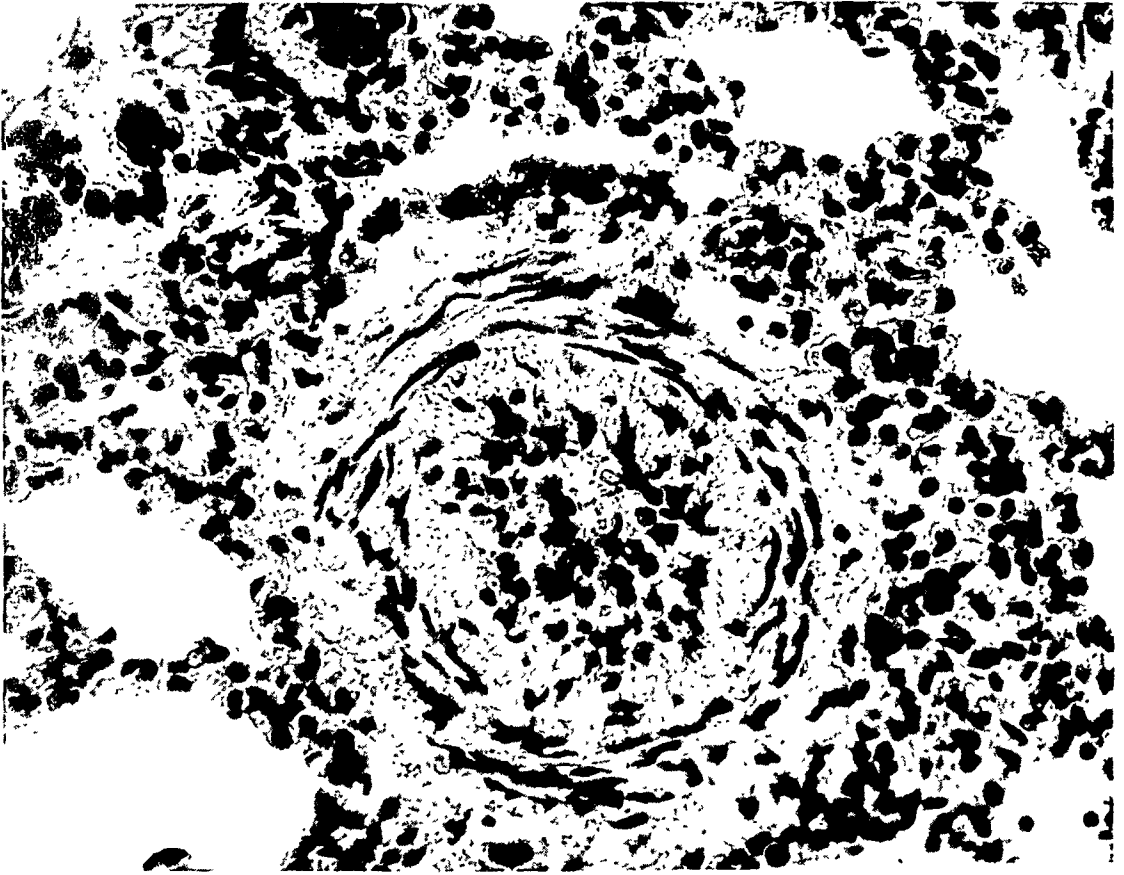


Fig. 7.—Photomicrograph of a small artery in a lung of rabbit 1, litter 9, showing acute purulent arteritis following inoculations with *Streptococcus anginosus* from a sphenoid sinus. There is purulent exudate in the lumen, infiltrating into the thickened intima. Note the loose fibrillar stroma in the adventitia.

a femoral hematoma and later a prolapsed rectum. All of our animals seemed to tolerate the inoculations without serious sequelae.

The monkeys after death showed no arteriosclerosis on careful gross examination. The changes observed in the various viscera were almost entirely microscopic, with the exception of fatty changes in some of the livers, which could be seen grossly. The microscopic changes included thickening of the walls of the pancreatic arteries in monkey 1. The pulmonary arteries in monkeys 3 and 4 had a lipoid infiltration and thickening of the intima, hypertrophy of the media and perivascular fibrosis. In monkey 5, the right femoral artery was thickened and surrounded by hyalinized muscle. In monkey 6, the right femoral artery

contained thickened intima and was surrounded by much scar tissue with obliterative arteriolitis in the surrounding tissue. The larger arteries of the kidneys contained thickened intimal linings. Monkey 7 showed a slight scarring around the right femoral artery. In addition to the arterial changes, monkey 1 presented accumulations of lymphocytes in the myocardium, subendocardium and subpericardium. Hyaline degeneration of the myocardium was observed about some of these foci. Similar changes were found in the hearts of monkeys 2, 6, 7 and 8. In the livers of monkeys 1, 2 and 6 there were marked accumulations of round cells in the periportal spaces, and in monkeys 2, 4, 6 and 8 there were focal and diffuse infiltrations into the interstitial tissue of the kidneys by lymphocytes.

#### COMMENT

It is unnecessary to review here the various attempts at experimental arteriosclerosis in animals. The important bibliography up to 1926 is included in a review by one of us (Benson).<sup>1</sup> The credit for first describing aortic lesions of rheumatic fever belongs to Klotz.<sup>2</sup> In 1926, von Glahn and Pappenheimer<sup>3</sup> made an exhaustive study of the changes in the aorta and other arteries in rheumatic fever. In a more recent publication<sup>4</sup> Pappenheimer and von Glahn described the intimal changes in a coronary artery, including a loose proliferation of endothelial tissue and the presence of an elevated parietal thrombus projecting into the lumen. Clawson<sup>5</sup> injected into rabbits and monkeys *S. viridans* from the blood of patients with rheumatic fever and subacute bacterial endocarditis, and obtained arteritis involving all coats. He considered this similar to the rheumatic lesions in man described by Klotz and by Pappenheimer and von Glahn.

Our observations on the aortic and other arterial changes bear a striking similarity to the descriptions that Clawson gave of his experimental lesions. Possibly, after all, there is a relationship between our experimental lesions, those described by Clawson, the rheumatic arteritis of Klotz and of Pappenheimer and von Glahn and the arteriosclerosis in the girl, 14 years old, referred to in our opening paragraph.

We have observed various stages of sclerosis in the aortas of inoculated rabbits in our series. The aorta shown in figures 4 and 8 had thickened intimal plaques composed of loose tissue containing irregular cells with deeply stained nuclei widely separated by fibrillar stroma

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1. Benson, R. L.: The Present Status of Coronary Arterial Disease, *Arch. Path.* **2**:876, 1926.

2. Klotz, O.: Rheumatic Fever and the Arteries, *Tr. A. Am. Physicians* **27**:181, 1912.

3. von Glahn, W. C., and Pappenheimer, A. M.: Specific Lesions of the Peripheral Blood Vessels in Rheumatism, *Am. J. Path.* **2**:235, 1926.

4. Pappenheimer, A. M., and von Glahn, W. C.: Studies in the Pathology of Rheumatic Fever, *Am. J. Path.* **3**:583, 1927.

5. Clawson, B. J.: Experimental Rheumatic Arteritis, *Arch. Path.* **6**:947, 1928.

having scattered clefts. A comparison of these photomicrographs with those of Pappenheimer and von Glahn<sup>6</sup> disclosed a close similarity. The intima of our aorta was comparable with theirs, but the media of ours was only slightly changed as compared with theirs. The aorta shown in figures 1 and 2 represented a more advanced stage of arteriosclerosis. The loose tissue of the intima was being replaced by fibrous

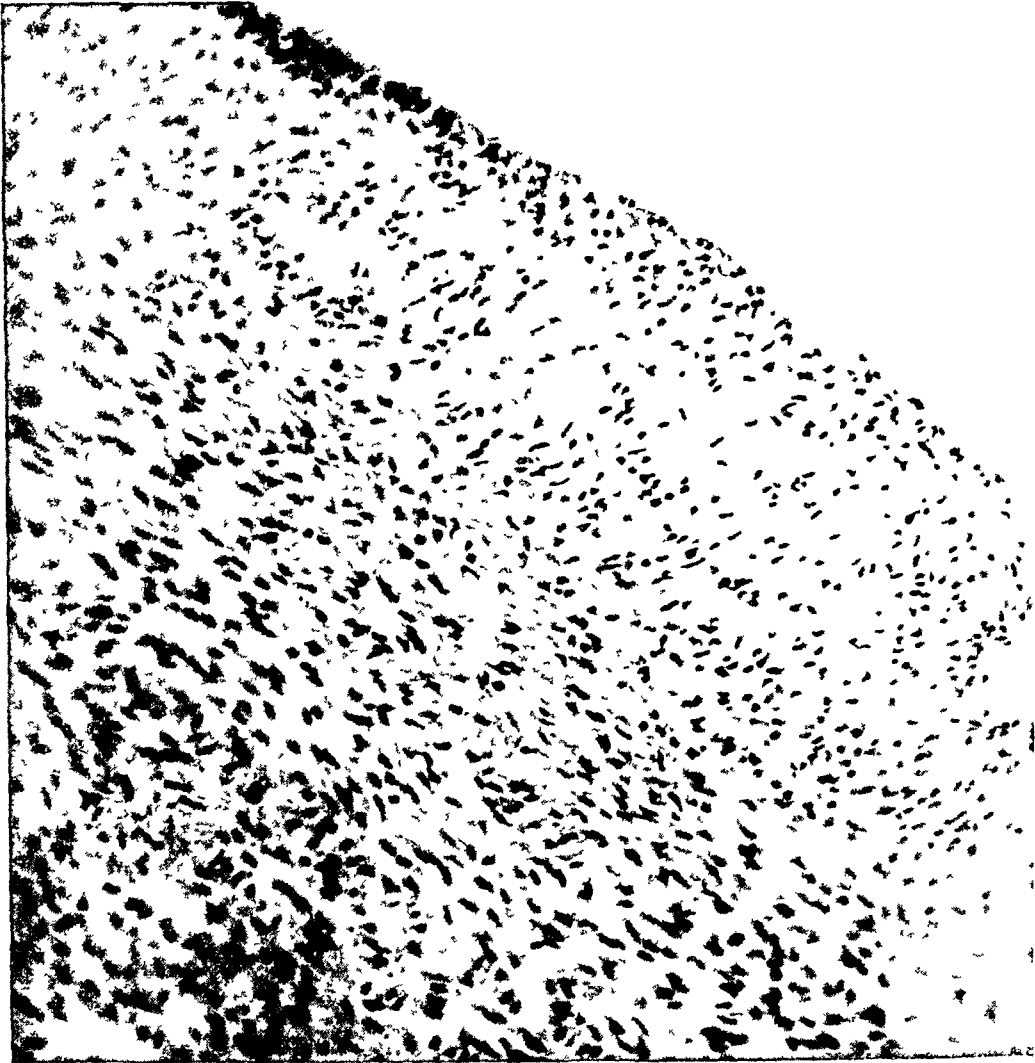


Fig. 8—Photomicrograph of the aorta of rabbit 3, litter 6, showing irregular intimal thickening in arteriosclerosis following inoculations with *Streptococcus viridans* from a coronary thrombus.

tissue, with resulting scar formation. Fibroblasts were numerous, and deeply blue-staining fibrillar strands traversed the scarred intima. The media exhibited numerous irregular and spindle-shaped, deeply staining

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6. Pappenheimer and von Glahn (footnote 4, plate 150, *A* and *B*, following p. 594).

cells, with hyaline change in the intervening tissue of the wall. The two aortas just referred to afforded instances of moderately early and fairly advanced stages of arteriosclerosis, respectively. Our other specimens varied chiefly in the extent of involvement and the stage reached.

Acute arteritis, sometimes purulent, occurred in some of the arteries. Figure 7 shows such a change in a small artery of the lung. The intima

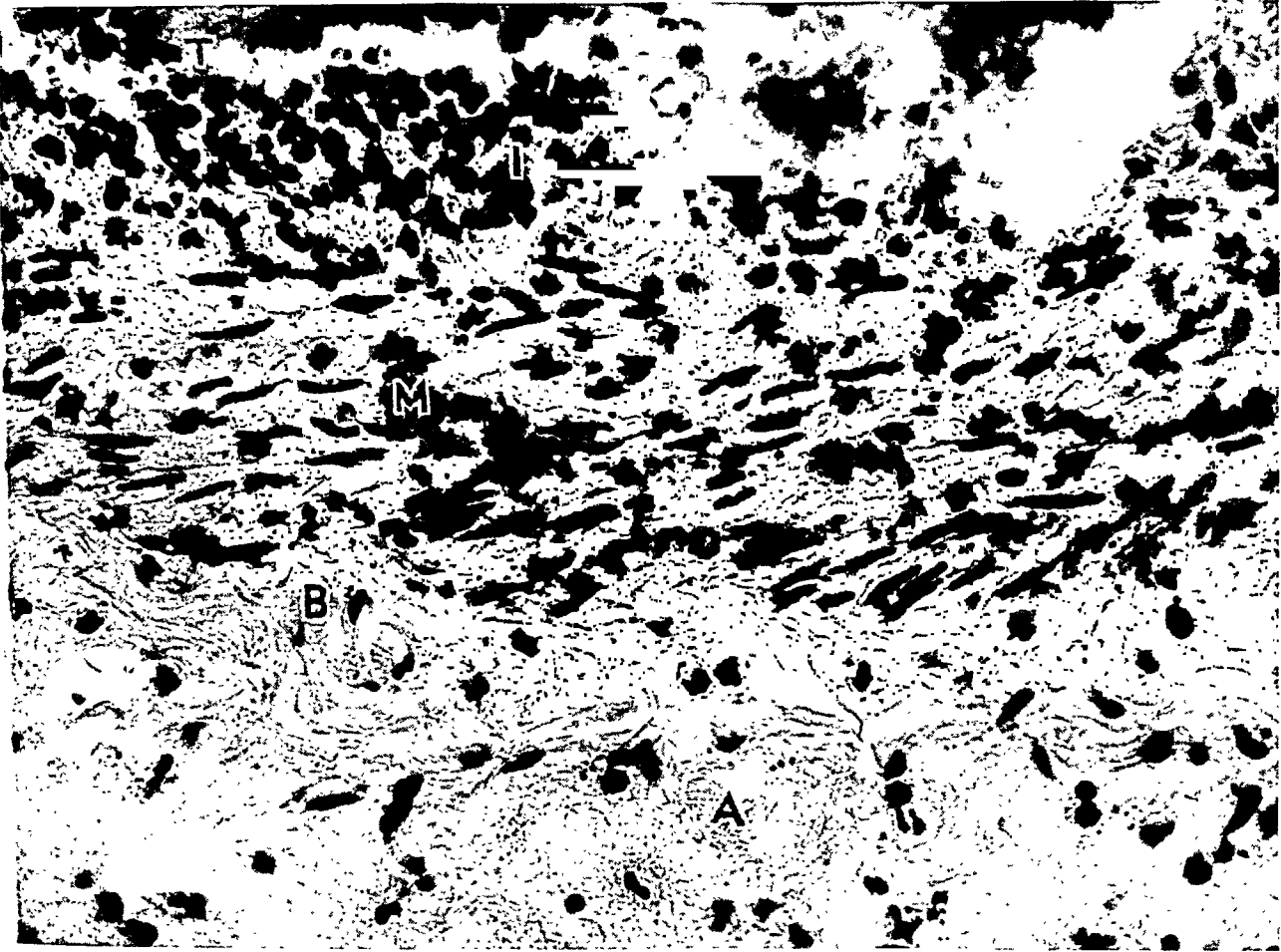


Fig. 9.—Photomicrograph of the wall of an artery in the lung, showing acute arteritis: *A*, the adventitia; *M*, the media with pyknotic nuclei; *I*, the purulent intima; *B*, bacteria in the wall; *T*, a thrombus in the lumen.

was thick and swollen and had a roughened surface with polymorphonuclear leukocytes and mononuclear cells attached to it. A mass of leukocytes and fibrin nearly filled the lumen, and polymorphonuclears were present in small number in the arterial wall. The adventitia consisted of a loose, relatively noncellular, fibrillar substance.

Purulent arteritis with an occluding thrombus was found in a large artery of one lung (fig. 9). The intima was thickened and densely



infiltrated with polymorphonuclears and fibrin, and this purulent exudate merged inseparably into a thrombus. Leukocytes were scattered throughout the wall, being especially dense in the adventitia. Bacteria could be seen in various parts of the wall, mostly diplococci.

Various stages of arteriosclerosis occurred. Figure 6 represents an early retrogressive stage with moderate intimal thickening characterized by loose, relatively noncellular tissue. Other arteries exhibited later stages of sclerosis with thickening of the entire wall, and with hyalinization.

In a review of series 3 and 4, we find that each of twenty rabbits was repeatedly inoculated with 1 cc. of streptococcus broth cultures, and that fourteen (70 per cent) acquired arterial lesions, of which those in seven were very definite on gross examination of the aorta. Thirteen of the twenty animals inoculated were fed on a normal diet, and eight of these (61 per cent) acquired arterial lesions. Nonhemolytic streptococci produced arteriosclerosis within three months in one rabbit, after twenty-three inoculations. The majority of the rabbits, however, were given twenty-seven inoculations each and were not examined until eight months had elapsed. Hemolytic streptococci were more virulent and killed most of litter 10 within one month. These animals had only five inoculations each, but showed abundant arterial changes. In litter 9 many survived ten inoculations and lived for nine months. Nearly all of the rabbits in this litter showed arteriosclerosis at the end of the experiment.

In the absence of gross aortic and arterial changes in series 5, we believe that our microscopic observations in monkeys are not conclusive.

*Influence of Cholesterol.*—The ingestion of an adequate amount of cholesterol for a sufficient length of time will produce arteriosclerosis in rabbits. Scarff<sup>7</sup> fed 50 Gm. of cholesterol, subdivided into daily doses of from 0.2 to 0.7 Gm., for six months before aortic changes appeared. Shapiro<sup>8</sup> found that thyroidectomy, splenectomy and gonadectomy facilitated experimental cholesterol arteriosclerosis.

We gave cholesterol feedings alone to one rabbit in all litters but one, and found no gross changes in the aorta after feeding a total of 65 Gm. of cholesterol over a period of nine months. In one rabbit, however, microscopic thickening of the arteries developed in seven months. From these findings we concluded that the quantity of cholesterol was not sufficient to create gross changes in the aorta. But when

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7. Scarff, R. W.: The Production of Experimental Atheroma with Cholesterol, *J. Path. & Bact.* **30**:647, 1927.

8. Shapiro, B.: The Influence of Thyroidectomy, Splenectomy, Gonadectomy, and Suprarenalectomy upon the Development of Experimental Atherosclerosis in Rabbits, *J. Exper. Med.* **45**:595, 1927.

the same quantity was fed to litter mates that received intravenous injections of streptococci, there was a marked involvement of the blood vessels. By combined bacterial injections and cholesterol feedings, arterial lesions were produced in six of seven rabbits (85 per cent) and gross signs of arteriosclerosis in the aortas of four of the seven (57 per cent). Arteriosclerosis was more prominent in these rabbits than in those without hypercholesteremia. These rabbits received an average of only 46 Gm. of cholesterol during an average period of seven months. The minimum number of inoculations with streptococci was five and the maximum number twenty-seven. It is probable that infection of the blood stream plays an important rôle in precipitating cholesterol in the inflamed walls of blood vessels.

*Spontaneous Arteriosclerosis in Rabbits.*—Sufficient evidence has accumulated in recent years to show that spontaneous arteriosclerosis, primary in the intima, is not common in normal rabbits. Newburgh and Clarkson<sup>9</sup> reviewed this subject and differentiated between true arteriosclerotic lesions of the intima and the spontaneous medial calcifications. Dominguez,<sup>10</sup> in 1928, summarized all of the available literature on spontaneous arteriosclerosis, and expressed the belief that the unusual incidence reported by Miles in 1907 was a local phenomenon. Since that time many normal animals have been examined in this country and in Europe, and in an analysis of the records of more than thirty-five hundred rabbits examined and reported on in the last twenty years, Dominguez found that by including all arterial changes the incidence was only 6 per cent, and of these only 4 per cent, or 0.3 per cent of the total number, showed severe lesions. Nuzum and co-workers<sup>11</sup> reviewed this subject in 1930 and studied one hundred and ninety rabbits living under normal conditions for two or three years. They concluded that spontaneous arteriosclerosis is infrequent.

All of our controls and inoculated rabbits were litter mates, and none of the controls had arterial lesions that were visible grossly. In the microscopic examinations, which were as thorough in the controls as in the inoculated animals, we found a spontaneously thickened artery in a control only once. Within two months this animal died of pneumonia and nephritis. The fact should be emphasized that our experimental animals all belonged to young, healthy litters and ranged between two and three pounds (907 and 1,360 Gm.) in weight at the beginning

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9. Newburgh, L. H., and Clarkson, S.: The Production of Atherosclerosis in Rabbits by Feeding Diets Rich in Meat, *Arch. Int. Med.* **31**:653, 1923.

10. Dominguez, R.: Effect on the Blood Pressure of the Rabbit of Arteriosclerosis and Nephritis Caused by Uranium, *Arch. Path.* **5**:577, 1928.

11. Nuzum, F. R.; Elliot, A. H.; Evans, R. D., and Priest, Blanche V.: The Occurrence and Nature of Spontaneous Arteriosclerosis and Nephritis in the Rabbit, *Arch. Path.* **10**:697, 1930.

of the experiments. All rabbits were examined by the end of ten months. In addition to our controls, we have examined more than one hundred uninoculated rabbits, exclusive of the present series, and have failed to find gross aortic lesions. We are convinced that spontaneous arterial lesions are rare in young healthy rabbits on a normal diet.

#### SUMMARY

We have described lesions in the aorta and other arteries in rabbits inoculated with streptococci from sclerotic coronary arteries, coronary thrombi and sinusitis. These lesions include acute artéritis with or without thrombosis and arteriosclerosis of the aorta and other arteries. The experimental changes are comparable with the corresponding arterial diseases found in man. Slight arterial changes were observed in monkeys following injections of streptococci, but the changes are regarded as inconclusive.

# HYPERVITAMINOSIS D AND ARTERIOSCLEROSIS\*

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Much experimental work has been done to determine the toxic effects on laboratory animals of excessive doses of viosterol (irradiated ergosterol). It has been shown that massive doses produce a marked metabolic disturbance characterized by a condition of hypercalcemia and hyperphosphatemia, and result in the formation of calcium deposits in various organs of the body. The recent work on viosterol has been summarized by Pfannenstiel.<sup>1</sup>

Ergosterol, the provitamin D, is a sterol obtained principally from yeast and ergot, and, when irradiated, has an antirachitic property 2,000 times that of cholesterol, in which substance ergosterol is found as an impurity (Blunt and Cowan<sup>2</sup>). Koch, Koch and Ragins<sup>3</sup> concluded, after experimenting with various sterols, that the provitamin D activity is not limited to ergosterol. The toxic effects of massive doses of viosterol have been reported on by Blunt and Cowan,<sup>2</sup> Duguid,<sup>4</sup> Spies and Glover,<sup>5</sup> Shohl, Goldblatt and Brown,<sup>6</sup> Klein<sup>7</sup> and others. Laas<sup>8</sup> in his experiments on rabbits gave single massive doses of viosterol of high potency, and observed vitamin D sclerosis of the aorta as early as the fifth day of the experiment. The altered phosphorus and calcium metabolism of animals treated with viosterol has been reported by Ashford,<sup>9</sup> Watchorn,<sup>10</sup> Brown and Shohl<sup>11</sup> and others. Rabl<sup>12</sup> found that sodium phosphate increased the toxicity of viosterol. The possibility has been suggested that vitamin D promotes calcification in

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\* From the Department of Pathology and Bacteriology, University of Toronto.

1. Pfannenstiel, W.: *Lancet* **2**:845, 1928.
2. Blunt, K., and Cowan, R.: *Irradiated Foods and Irradiated Ergosterol*, J. A. M. A. **93**:1301, 1929.
3. Koch, F. C.; Koch, E., and Ragins, I. K.: *J. Biol. Chem.* **85**:141, 1929.
4. Duguid, J. B.: *J. Path. & Bact.* **33**:697, 1930.
5. Spies, I. D., and Glover, E. C.: *Am. J. Path.* **6**:485, 1930.
6. Shohl, A. T.; Goldblatt, H., and Brown, H. B.: *J. Clin. Investigation* **7**: 505, 1930.
7. Klein, I. J.: *Effects of Massive Doses of Irradiated Ergosterol; Preliminary Report*, J. A. M. A. **92**:621, 1929.
8. Laas, E.: *Virchows Arch. f. path. Anat.* **278**:346, 1930.
9. Ashford, C. A.: *Biochem. J.* **24**:661, 1930.
10. Watchorn, E.: *Biochem. J.* **24**:631, 1930.
11. Brown, H. B., and Shohl, A. T.: *J. Biol. Chem.* **86**:245, 1930.
12. Rabl, C. R. H.: *Deutsche med. Wchnschr.* **55**:63, 1929.

the body through a stimulative action on the parathyroid glands. It is notable that the toxic symptoms and the sites of deposition of calcium in tissues of dogs following treatment with parathyroid extract described by Learner<sup>13</sup> and Hueper<sup>14</sup> are similar to those in animals as a result of hypervitaminosis D. Morgan and Garrison<sup>15</sup> studied the effect of vitamin D and the reaction of the diet on the response to parathyroid extract, and concluded that vitamin D had an intensifying effect on the toxic action of parathyroid extract, also that vitamin D and an alkaline reaction of the diet, together or separately, increase the response to parathyroid extract. Pappenheimer<sup>16</sup> stated that cod liver oil and viosterol in therapeutic doses are both antirachitic in the absence of parathyroid gland or of thymus, or of both. The purpose of this study has been to produce arteriosclerosis in rabbits by means of large doses of irradiated ergosterol, and to follow the sequence of events in the pathologic processes in the affected vessels; and also to ascertain the order of involvement of tissues other than arteries that shown evidence of calcareous degeneration as a result of hypervitaminosis D.

#### THE EFFECT ON RABBITS OF THE ADMINISTRATION OF TOXIC DOSES OF IRRADIATED ERGOSTEROL

*Materials and Methods Used.*—For these experiments twenty-four young healthy rabbits were selected, ranging in age from 3 months to 1 year. For treatment with irradiated ergosterol, these were divided into small groups according to their age and weight. The irradiated ergosterol used was a biologically tested product with a potency of 10,000 D;<sup>17</sup> therefore, the vitamin D potency was 10,000 times the vitamin D potency of good cod liver oil. The dosage selected for the year old rabbits was approximately 0.5 cc. per kilogram of body weight, and the dosage for the younger animals was varied proportionately. In all the experiments the irradiated ergosterol was given in biweekly doses by the subcutaneous route, sterile precautions being used, and the termination of each experiment was by the spontaneous death of the treated animal. At autopsy, after macroscopic examination, blocks of tissue from the following organs were immediately preserved in a neutral solution of formaldehyde, U. S. P. (1:10), and in Zenker's solution: aorta (complete) and branches, heart, kidneys, liver, lung, stomach, spleen, pancreas, suprarenal glands, brain, thyroid gland, parathyroid and salivary glands and bone marrow of the femur. After fixation, frozen and paraffin sections were made. Frozen sections were stained with sudan III to demonstrate fat, and counterstained with hematoxylin. The principal stains used for the paraffin sections were hema-

13. Learner, A.: J. Lab. & Clin. Med. **14**:921, 1929.

14. Hueper, W.: Metastatic Calcifications in the Organs of the Dog After Injections of Parathyroid Extract, Arch. Path. **3**:14, 1927.

15. Morgan, A. F., and Garrison, E. A.: J. Biol. Chem. **85**:687, 1930.

16. Pappenheimer, A. M.: J. Exper. Med. **52**:805, 1930.

17. Viosterol 10,000 D was supplied for the experimental work by Mead, Johnson and Company.

toxylin and eosin, Verhoeff's method for staining elastic tissue, van Gieson's picrofuchsin, alizarin and von Kossa's silver nitrate method for identifying calcium deposits.

*Macroscopic Observations.*—The general effects noted in the rabbits receiving large doses of irradiated ergosterol were a continuous loss of weight and finally cachexia followed by death. At autopsy, there was no gross lesion indicating the cause of death; the animals were poorly nourished, and there was a noticeable absence of adipose tissue.

The aorta, when marked medial involvement was present, showed an irregularity of contour from an outpouching and twisting of its walls. Such an aorta, when cut open, revealed a multitude of lesions that were visible on its intimal surface as grayish, plaquelike depressions varying in size and bounded by the normal arterial wall, which had the appearance of a fold in this situation. The findings varied in the different animals, from a minor degree of arterial involvement situated about the aortic sinuses, to a diffuse embedding of the calcareous deposits in the aortic wall and its immediate branches.

The heart in the longer experiments was generally hypertrophied—in all cases the walls were relaxed and flabby. Except in one animal, the cut surface always appeared normal. In this case (no. 84) the heart, when cut open, showed a grayish-white area, irregular in outline, which extended through the interventricular septum. Later this proved to be an area of calcification. In two cases (nos. 84 and 182), the anterior surface of the pericardium had a wrinkled, withered appearance, and this portion was shown in stained sections to have undergone a hyaline change.

The kidneys were normal in shape; occasionally they were congested and soft. The capsules stripped readily, leaving a glistening, smooth surface. The cut surfaces of these kidneys presented no gross abnormalities; only occasionally a minute area of calcification was visible.

In a few animals a pneumonic condition was evident in the lungs. Frequently the lower borders of the lungs were congested. In one case (no. 182), a section of the pleural surface appeared similar to the pericardial surface mentioned—dry and wrinkled, and somewhat rough to the touch. Microscopically, this part of the membrane showed a calcium deposit covering its surface, while in the adjoining sections a hyaline change had occurred.

The liver was usually normal in appearance, unless the rabbit was infected with coccidia. The liver of one animal (no. 138) had an entire lobe calcified; this lobe was attached to the normal-appearing liver by a firm, fibrous pedicle.

The spleens of these animals were, as a rule, much smaller than normal and appeared congested.

The bone marrow of the femur was soft and varied from pale to deep red.

The stomach in one animal had a firm, raised, yellowish-white area in its wall; this region was later shown to contain calcium. No other abnormalities were noted.

The other organs examined appeared normal.

*Microscopic Observations in Arterial Lesions.*—The type of arteriosclerosis found in the arteries of rabbits treated with toxic doses of irradiated ergosterol is comparable to that produced in rabbits by the administration of epinephrine hydrochloride, barium chloride, digitalin and diphtheria toxin, as described by Klotz,<sup>18</sup> and to the experimental work-arteriosclerosis described by the same author,<sup>19</sup> which resembled the Mönckeberg sclerosis of the peripheral arteries in human beings, the main points of difference between vitamin D sclerosis and the Mönckeberg type being the site of the earliest lesions, which will be described later. There is a general agreement among experimenters as to the type and histologic characteristics of the advanced vitamin D lesion, but more differences of opinion are expressed about the earliest site of arterial involvement. The variation in the early observations reported by different authors is possibly due partly to the fact that individual animals of the same species vary in their susceptibility to the toxic action of viosterol, and to the variation in potency of the preparations of irradiated ergosterol used in their experiments (Hess, Lewis and Rivkin<sup>20</sup>).

In describing arterial lesions found in rabbits it is well to keep in mind the fact that this animal is subject to spontaneous sclerosis, which may attack both the intimal and the medial coats of the blood vessel, and which manifests itself as a proliferative lesion in the intima and a degenerative one in the media (Nuzum, Elliot, Evans and Priest<sup>21</sup>). The medial lesions occurring in rabbits treated with viosterol differ from the Mönckeberg type of arteriosclerosis both in the fact that the muscle cells are not the earliest site of the calcareous deposit in these arteries, and in the fact that the middle zone of the media is not the area commonly involved in the mature lesion. The earliest calcareous deposits are intimately related to the elastic fibers in the aorta and to the internal elastic lamina of the muscular type of arteries, and in both types of vessel to the interstitial substance adjacent to the involved fibers. The location of the early lesions in the aorta tends

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18. Klotz, Oskar: Brit. M. J. **2**:1767, 1906.

19. Klotz, Oskar: Montreal M. J. **37**:165, 1908.

20. Hess, A. F.; Lewis, J. M., and Rivkin, H.: Newer Aspects of Therapeutics of Viosterol (Irradiated Ergosterol), J. A. M. A. **94**:1885, 1930.

21. Nuzum, F. R.; Elliot, A. H.; Evans, R. D., and Priest, B. V.: The Occurrence and Nature of Spontaneous Arteriosclerosis and Nephritis in the Rabbit, Arch. Path. **10**:697, 1930.

to be along the inner half or inner third of the arterial wall; in the arch, calcareous plaques are frequently scattered throughout the medial coat, while on the lower section of the abdominal aorta the lesions lie to the inner third of the media, where, without exception, they are found in early lesions of the muscular type of arteries. In the experiments concerned here, only in one artery was evidence found of a secondary reaction, proliferative in nature, occurring in the intima over an underlying area of medial degeneration. Intimal proliferation was more common in relation to a hypertrophied section of the media in which no other pathologic change was demonstrable. In the late stage, the medial lesions were degenerative, causing such destruction to the medial coat that at the site of extensive calcification this coat appeared markedly thinned, while externally the vessel showed small



Fig. 1 (experiment 356).—Arch of aorta showing vitamin D arteriosclerosis. Note calcareous deposits about elastic fibers in the media. Hematoxylin and eosin;  $\times 80$ .

outpouchings of its wall. The explanation of these apparent dilations was found on microscopic examination of the cross-sections of the walls of the vessel. It was then noted that in these areas the elastic fibers were straightened out and rigidly fixed in an extended position by calcareous granules deposited over the elastic fibers and in the tissues about them; therefore, these portions of the wall of the vessel could not undergo the usual postmortem contraction. On the intimal surface, these lesions appeared as depressed areas varying in circumference from 0.1 to 0.4 cm., with their margins thrown up into a fold. The intima over them appeared unchanged.

In the aorta, the earliest calcareous deposits appeared focally in the cement substance along the surface of the elastic fibers of the media, or in the intercellular matrix lying immediately adjacent to these fibers, so that they first appeared to be outlined with a fine, granular



deposit, which later took the form of an incrustation. These granules stained a deep purple with hematoxylin, and a color varying from brown to black with silver nitrate, a finding that has been accepted in the past as representing a deposit of calcium salts in the tissues. Recently Cameron<sup>22</sup> reported that there is no specific stain for calcium, and that areas of calcification are very complex in structure, with calcium occurring only as a small portion of the deposit. Preceding the appearance of the granular deposit, a change took place in the intercellular matrix; there was an increase of this substance, produced apparently by some process of dilution, whereby its staining qualities



Fig. 2 (experiment 355).—Iliac artery showing calcareous deposit in the cement substance along the medial border of the internal elastic fiber. Hematoxylin and eosin;  $\times 200$ .

were altered so that the areas stained faintly or not at all. This change was neither a mucoid nor a fatty degeneration.

The incrustation of short lengths of elastic fibers mechanically interfered with the pliability of these fibers, so that the contraction waves became coarse and irregular; adjacent affected fibers appeared to increase in length and tended to contract in unison and later straighten out and lie in parallel lines in the axis of the circumference of the media. Some of the fibers were frequently pushed widely apart in an uneven manner owing to the increase in the intercellular

22. Cameron, J. R.: *J. Path. & Bact* **33**:929, 1930

substance and also to the contraction of the adjacent muscle fibers. Later there was evidence of a crowding together of the affected fibers away from the direction of the lumen of the vessel.

At this stage, the muscle cells lying between the involved elastic fibers still appeared healthy and stained normally, while the elastic fibers themselves were unaltered and stained evenly by Verhoeff's method. In more advanced lesions, the staining of the elastic fibers became interrupted at varying intervals and later the stained fibers presented a granular appearance. The muscle cells became compressed by the

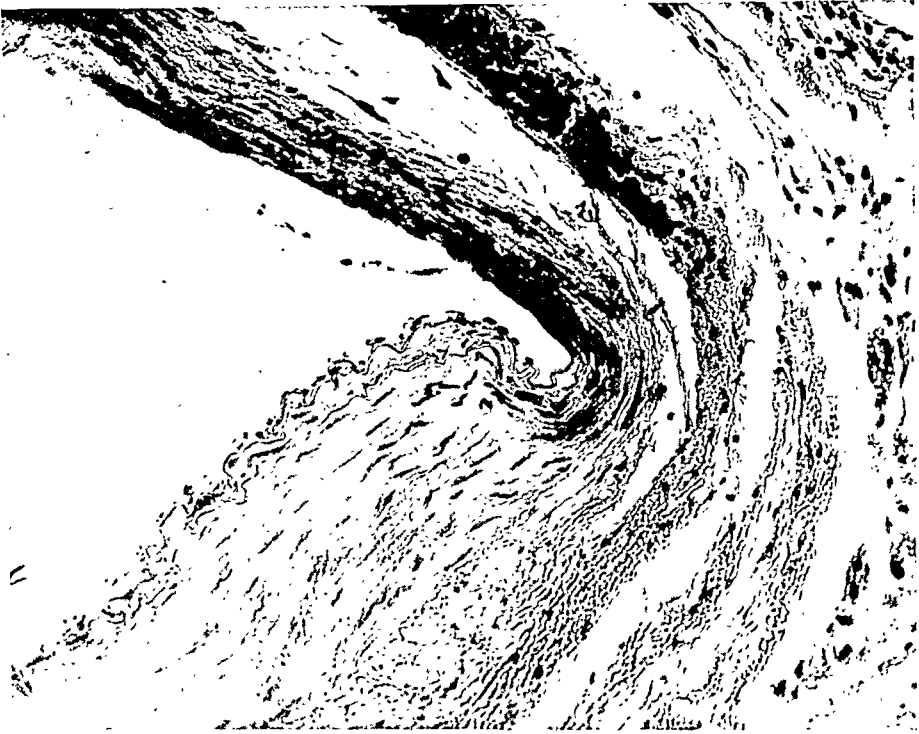


Fig. 3 (experiment 138).—Renal artery showing calcification of media and adventitia in one portion, and elastic laminae closely compacted. Hematoxylin and eosin;  $\times 200$ .

crowding together of the elastic fibers, and the cytoplasm and nuclei of the thin, elongated cells stained deeply. Later, when a heavy, plaque-like deposit of calcium was formed in the interstitial substance, the muscle cells in the central part of the lesion underwent degeneration, the cytoplasm of each cell becoming swollen and vacuolated with a stringy appearance of the cell margin. The nuclei were small and pyknotic. Following this change, the muscle cells became necrotic, the elastic fibers atrophied and were broken up into small fragments, and a coarsely granular mass of calcareous material filled the area of degeneration. The margins of these lesions shaded off into normal tissue. It is surprising what an accumulation of calcareous material

is first deposited over and about the elastic fibers and muscle cells, before either show evidence of marked degeneration.

In the muscular arteries, the sequence of arteriosclerotic change was similar to that found in the elastic type of blood vessel. The earliest site of involvement was the medial side of the elastic lamina. Here a focal change occurred in the interstitial substance, revealed by an increase in its volume and a decrease in the intensity of its staining qualities. Accompanying this process, a fine, granular deposit of calcium was formed along the medial surface of the internal elastic lamina, at first as a thin covering, and later as a dense coat surrounding it for

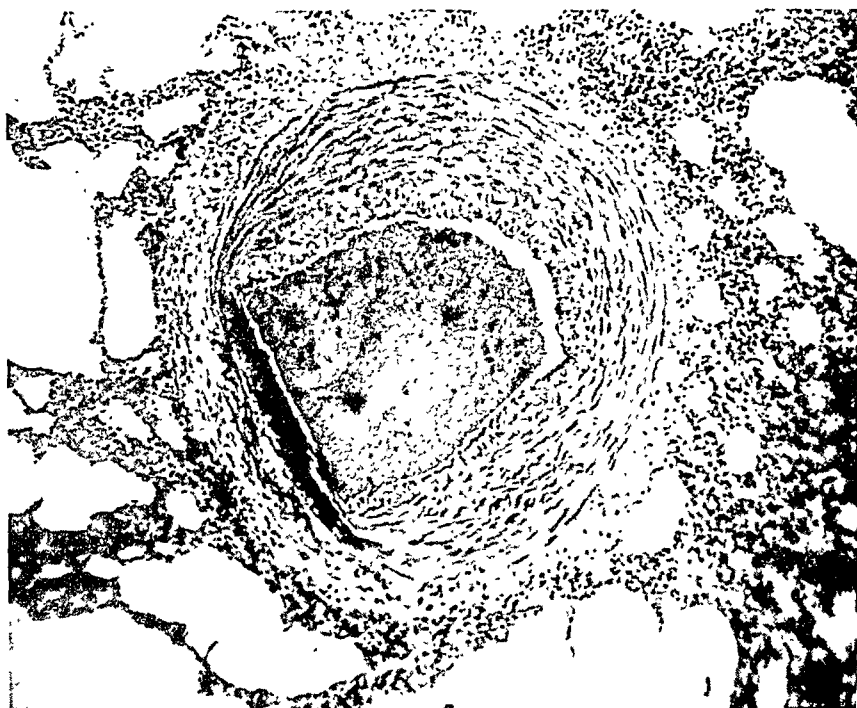


Fig. 4 (experiment 182).—Bronchial artery showing calcification of one segment of the media, while the remaining portion is hypertrophied. Hematoxylin and eosin;  $\times 80$ .

short intervals. This deposit spread in the adjacent intercellular substance, until at a late stage the entire media of a segment of the wall of the vessel was involved, the adventitial coat rarely being included. None of the lesions in the muscular arteries showed evidence of a degenerative change in the internal elastic membrane. There was only a mechanical interference with its pliability, shown by a straightening out of the usual fine contraction waves so that the membrane was thrown into coarse, irregular folds when the underlying active muscle cells contracted. The muscle cells in these arteries were compressed from the direction of the lumen of the vessel outward, but except for

small areas showing a hyaline-like change where this compression was most marked, no primary degeneration of the muscle cells occurred. The cells lying just external to the internal elastic membrane were the first to be caught in the outward extension of the calcium deposit from the region of the lamina, and thus lost their contractile power and followed the uneven folds of the lamina, while the more external, healthy muscle fibers continued to contract in a normal manner.

The apparent thinning of the wall of the vessel in early vitamin D lesions before definite degeneration of the tissue elements takes place is a mechanical phenomenon due to pressure from within the lumen of the vessel outward, first manifested by the compression of the muscle cells in the layer situated directly external to the inner elastic lamina, and gradually involving the entire medial coat. Evidence that the inner coats are most affected by a process of stretching and loss of elasticity of their fibers is found in the aorta studded with vitamin D lesions which, when cut open, everts in both a longitudinal and a horizontal direction.

In this series of experiments, intimal lesions were uncommon as a result of hypervitaminosis D. The lesions found in the intima were minute and almost without exception hyperplastic, produced by a splitting of the internal elastic membrane and an increase of the muscle cells and fibrous tissue. In all these cases, the underlying arterial wall showed no evidence of degeneration or calcification, but instead, a marked hypertrophy of the muscle cells and an increase in the number of elastic fibers. These lesions suggested a compensatory hyperplasia of the intima produced by increased functional activity, the same stimulus as that affecting the cell activity of the underlying medial coat. Increased work over an extended period has been found to cause hypertrophy of the musculo-elastic layer of the intima and splitting of the internal elastic membrane in carotids of experimental animals (Klotz<sup>23</sup>). One animal showed a more advanced lesion of this type; in this, the internal elastic lamina was split into numerous fibers, which were spread apart by proliferating layers of muscle cells. In the resulting nodule formation, many of the muscle cells were rounded in outline with clear, vacuolated cytoplasm, containing centrally placed pyknotic nuclei. Varela<sup>24</sup> and his co-workers described this type of lesion as one specific for vitamin D sclerosis.

As mentioned before, in one animal only was there found a small regenerative lesion of the intima, which occurred over an area of extensive medial calcification. Duguid<sup>4</sup> from his experiments reported hyperplasia of the intima only over areas of medial degeneration.

23. Klotz, Oskar: *J. Exper. Med.* **12**:707, 1910.

24. Varela, B.; Collazo, J. A.; Moreau, J., and Rubino, P.: *Virchows Arch. f. path. Anat.* **274**:270, 1929.

Evidence of a toxic degeneration of the muscle cells occurred in focal areas in portions of the aorta where there was no suggestion of a calcareous lesion. The injured cells were from round to oval in outline, with clear, colorless cytoplasm and contained centrally placed, pyknotic nuclei. In frozen sections, these cells showed no evidence of fat. These lesions occurred in the outer or inner third of the medial coat or throughout the entire medial coat. Laas<sup>8</sup> found the same type of degeneration in rabbits exposed to chronic intoxications, particularly in animals that had died a natural death. In the present experiments, the same type of degeneration was found occurring in muscle cells situated in the longitudinal muscle layer of the stomach.

TABLE 1.—*Relation of Length of Experiment to Degree of Calcification of Aorta*

Experiment	Days	Gain or Loss in Weight, per Cent	Calcification of Aorta	Age of Rabbit, Mo.	Total Dosage of Viosterol 10,000 D, Cc.
353	18	—15.7	0	12	0.5
356	25	—31.9	+	12	1.1
354	28	—42.6	++++	12	3.6
355	33	—20.4	++++	12	4.6
352	42	—32.9	+	12	6.6
357*	70 (killed)	+ 6.1	0	12	7.1
139	14	—17.5	++++	12	2.0
178	30	—34.1	++++	12	8.0
138	41	?	++++	12	6.0
182	62	—37.2	++++	12	17.0
11	11	?	++++	3	2.0
9	16	?	0	3	2.5
10	25	—37.0	+++	3	3.5
12	25	—38.0	+++	3	3.5

\* The animal in experiment 357 received calcium (in the form of calcium gluconate-Sandoz) in daily doses of 15.4 mg. intramuscularly. The animal gained in weight and was killed on the seventieth day of the experiment. No areas of calcification were found in the tissues.

The occurrence of the calcareous lesions in the inner thirds of the arteries has been referred to. The region of the aortic sinuses was the first area attacked. The process extended downward, and no segment of an artery within the involved area was found free. The process frequently stopped abruptly at the diaphragm, or extended along the abdominal aorta to its branches and to the vessels of the extremities. There was no evidence of grouping of the calcareous plaques about the ostia of the intercostal arteries or the lower branches of the aorta. There was a tendency for the lesions to miss the under part of the aortic arch and the posterior wall of both the thoracic and abdominal aorta. The most severe changes were in the arch and thoracic aorta. The cardiac valves and the pulmonary vein were frequently extensively calcified.

Fatty changes were not present in all lesions of medial calcification produced by viosterol. In frozen sections stained with sudan III it was usual to find from fine to coarse droplets of fat between the elastic fibers in the medial coat surrounding a thick, calcareous plaque. The droplets tended to mass in the tissues lying at the external margin of the calcified area. These observations were not constant, for often in an adjacent lesion of apparently equal intensity no fat could be demonstrated; nor was evidence of fat found in relation to the early granular deposits in the media. After decalcification of frozen sections, the site of a previously heavy calcareous deposit and the external border of such a lesion often showed fine droplets of pale yellow-staining fat, or coarse fat globules, located in the interstitial substance lying between

TABLE 2.—*The Effect of Excessive Doses of Viosterol on Rabbits*

Treatment	Experiment	Days	Total Dose, Cc.	Initial Weight Gm.	Weight Loss, per Cent	Weight						
						Aorta	Muscular Arteries	Heart	Lungs	Kidneys	Stomach	Liver
Viosterol 10,000 D, biweekly dose 0.1-1.0 cc.	354	28	3.6	1,830	42.6	++++	++	+	0	+++	Trace	0
	178	30	8.0	2,430	34.1	++++	+	0	0	++	++++	0
	355	33	4.6	1,565	20.4	++++	+++	Trace	0	++	++	0
	182	62	17.0	2,150	37.2	++++	+++	0	++	Trace	0	0
Viosterol 10,000 D, biweekly dose 0.1-0.5 cc.	139	14	2.0	2,000	17.5	++++	++	+	++	++++	+++	0
	356	25	1.1	985	31.9	+	0	0	0	Trace	0	0
	10	25	3.5	875	37.0	+++	0	0	0	++	+++	0
	12	25	3.5	810	38.0	+++	+	Trace	Trace	+	0	0
	135	41	6.0	2,000	?	++++	++++	0	0	+	++	+++
	352	42	6.6	940	32.9	+	0	0	0	0	0	0

+ = degree of calcification of tissues.

the elastic fibers. In the intima, fat droplets were occasionally present within the cells of a proliferative lesion.

*Calcification in Organs Other Than Arteries.*—An extensive deposit of calcium in the cardiac muscle occurred in one animal in an area of degenerated tissue surrounded by a zone of infiltrating leukocytes and walled off by fibrous tissue. In three other animals, minute deposits of calcium were found in small focal areas of degenerated muscle where the muscle fibers were vacuolated. In these four cases, the coronary arteries were moderately sclerosed. In two other cases there was a hyaline degeneration of a portion of the pericardium.

The kidneys of eight animals showed calcareous deposits in the parenchyma and slight deposits in some of the blood vessels. The lesions varied in severity; only in one case was there marked involvement of the organ. Calcium casts occurred, as a rule, in the tubules of the cortex and frequently were associated with further destruction

of the tubular epithelium in the areas where these casts had lodged. Calcium deposits were found in the basement membrane of scattered tubules in the cortex, also rarely in the glomerular capsules and in the media of the interlobular arteries and arterioles. In four of these cases, the renal artery was sclerosed. Sections of kidney stained for fat showed heavy fat deposits in the degenerating tubules that were filled with casts of calcareous material. In all the kidneys there was a granular degeneration of the tubules, generally accompanied by cloudy swelling.

Two animals showed calcium deposits in the lungs; in one of these there were a few scattered granular particles in the mucous membrane of a large bronchus; in the other (no. 182), the greater part of the pleura of one lobe, which was covered on its inner surface by an extensive inflammatory exudate, was completely calcified. Areas of the pleura not calcified had undergone hyaline degeneration. Calcification had also occurred in the elastic fibers in the wall of a large bronchus. The bronchial arteries, the pulmonary vein and the smaller vessels were markedly sclerosed. Both these animals had an inflammatory process in the lung simulating bronchopneumonia. No calcification occurred in the bronchial muscles in any of the animals.

Calcification occurred in the livers of three animals. In two, the process concerned greatly enlarged bile ducts, the result of infection with coccidia. The third animal had one lobe of the liver calcified, as mentioned before. Examination of the adjacent, fairly normal tissue leading to the pedicle of the calcified lobe showed marked destruction of the liver parenchyma and fibrosis, but no indication of whether the disturbance was a vascular or an inflammatory one. In every case some form of tissue degeneration was present in the liver. Congestion was generally marked, with varying degrees of atrophy of cells about the central areas.

In six animals, the stomach showed calcification of a portion of its wall; in four, moderate calcareous deposits were located in the circular muscular coat; in one, a small amount of calcium was deposited in the longitudinal muscle fibers, with a few scattered granules in the glands of the mucous coat; the remaining animal had a moderate deposit only in the serosa, and in this area there was a small sclerosed artery. These calcareous deposits in the muscle fibers were found only in regions of focal degeneration, which were scattered through the muscle coats. The cytoplasm of the muscle cells in these situations first appeared vacuolated; later a fine granular deposit of calcium was laid down either in the degenerating cells or in the intercellular substance about their borders.

Brain tissue showed no abnormalities and no sclerosis of the arteries.

The spleen in the majority of cases was markedly congested and frequently showed large amounts of hemosiderin phagocytosed by endothelial cells. The malpighian bodies were degenerated and atrophied, and diminished in number, so that often few corpuscles remained in the degenerated pulp tissue. In one case, the splenic artery was sclerosed.

No calcified areas were found in the suprarenal glands. In one gland a small, oval section of bone complete with a centrally placed medulla was incorporated in the cortex just beneath the capsule. There was no disturbance of the surrounding parenchyma.

The thyroid glands indicated various states of activity. Two were extensively fibrosed; the others appeared normal.

There were no gross abnormalities in the parathyroid glands; they were about normal in size or showed a very slight increase above the normal, except in one case in which the glands were definitely enlarged. The parenchymal cells were normal in size, shape and staining qualities and in relation to each other. In these parathyroid glands the capillary channels were distended and congested. In dogs dying of acute hyperparathyroidism, the parathyroid glands are reduced to one-half their size, and the parenchymatous cells are distorted and diminished in size (Jaffe and Bodansky<sup>25</sup>).

The salivary glands were normal. The pancreas in the majority of cases indicated no abnormality; however, in three cases the islet cells had undergone degeneration; the cytoplasm was granular and occasionally vacuolated, the cell margins were indefinite, and many of the nuclei were small and stained deeply.

The bone marrow in every case showed a marked hyperplasia of the erythrogenic centers, with congestion of the capillaries, usually accompanied by an increase in the number of granular cells. There was no evidence of destruction of marrow or of fibrosis such as that produced by experimental hyperparathyroidism (Jaffe and Bodansky<sup>25</sup>).

Rabbit 357 received a total dosage of viosterol that exceeded any other dose administered in the same experimental group, together with daily doses of calcium gluconate. I cannot give any explanation why this animal, when killed on the seventieth day of the experiment, showed no evidence of calcareous lesions either in the arteries or organs.

#### CONCLUSIONS

Vitamin D arteriosclerosis is not a specific type, for well developed lesions resemble those experimentally produced by the administration of epinephrine hydrochloride, barium chloride and digitalin, the main point of difference being the primary site of tissue involvement. Inflam-

25. Jaffe, K. L., and Bodansky, A.: *J. Exper. Med.* **52**:669, 1930.



matory processes are not a factor in its production, as no cellular infiltration occurs in or about these lesions. Nutritional disturbances alone do not account for their early formation, for instead of the entire arterial wall becoming involved, the lesions are patchy in their distribution and appear to follow the location of areas of stress and strain in the vessel. Evidently the toxic and mechanical factors are more important. Toxic degeneration occurs in these arteries, and though not always in relation to the primary medial calcification, it appears to have a relation to the changes in the intercellular substance that allow calcium to be deposited in that region. No specific type of degeneration of tissue precedes the initial deposit of calcium about the elastic fibers. At a later period, the mechanical factor introduces its local influence. Evidence occurs in the tissues of the failing ability of the arteries to maintain a proper circulation in the body. At autopsy, the heart is hypertrophied, there are passive congestion of the spleen and focal necrosis in the liver, congestion of the lungs and of the capillaries of the bone marrow, and generalized hypertrophy of the walls of the smaller blood vessels.

The first action of vitamin D on the tissues of organs other than blood vessels appears to be a toxic one, as shown by the focal areas of loosening of the muscle cells from their embedding substance, so that they tend to assume a rounded outline and later undergo degeneration and calcification. This is a prominent observation in the cardiac muscle and the muscle of the wall of the stomach. The early toxic action on the renal epithelium is also marked by an extensive granular degeneration and cloudy swelling. The calcium deposits in the organs due to hypervitaminosis D are, in practically every case, preceded by demonstrable injury to the tissue. Calcification of the muscles of the heart and stomach is preceded by a specific type of degeneration, whereas the type of change leading to calcification of the arterial walls is less definite. In the heart, as these experiments showed, calcification is preceded by sclerosis of the coronary arteries and the degeneration of small focal areas of muscle fibers, generally located near sclerotic blood vessels.

In the kidney, the first sign of calcification is the appearance of calcareous casts formed around degenerated epithelial cells and débris in the tubules. Later the tubular epithelium opposite the localization of the casts undergoes degeneration and calcification. Degeneration of the renal parenchyma occurs in relation to sclerosis of the interlobular arteries and their branches. This is followed by calcification.

It would appear that calcium deposits occurring in hypervitaminosis D have a predilection for elastic tissue, as evidenced by the regularity of the deposits along the elastic fibers in the blood vessels and the

involvement of the elastic tissue of the walls of bronchi, as well as for tissues suffering hyaline degeneration.

The earliest changes in elastic fibers are difficult to demonstrate, so that it is possible that these fibers undergo some type of degeneration prior to the deposition of calcium along their outer surfaces.

In these experiments there was no indication that excessive doses of irradiated ergosterol cause any morphologic change in the parathyroid glands of the rabbit, either in the form of a hyperplasia or of a degenerative process in the parenchyma of the glands.

#### SUMMARY

The arteriosclerosis produced by hypervitaminosis D is not specific.

The earliest calcareous deposits in the blood vessels are in the form of an incrustation about the elastic fibers in the media, and are preceded by some change in the adjacent intercellular substance. Degeneration of the muscle cells of the media appears at a later period.

In these experiments, the order of involvement of tissues showing calcareous deposits as a result of hypervitaminosis D, other than arteries, was: kidneys, stomach, heart, liver and lungs.

Tissue degeneration due to a toxic process precedes calcification of these organs.

The proliferative lesions occurring in the intima in arteries are not dependent on a primary degeneration of the media.

No changes occurred in the bone marrow or in the parathyroid glands comparable to those found in hyperparathyroidism.

# EARLY CELLULAR REACTION TO TUBERCLE BACILLI

A COMPARISON OF THIS REACTION IN NORMAL AND TUBERCULOUS  
GUINEA-PIGS AND IN GUINEA-PIGS IMMUNIZED  
WITH DEAD BACILLI \*

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The character of the cellular response to infection with tubercle bacilli has been the subject of long continued active investigation. Especial emphasis has in recent years been laid on the monocyte, which Cunningham, Sabin and their co-workers<sup>1</sup> identified as the precursor of the epithelioid cell. Sabin, Doan and Forkner,<sup>2</sup> moreover, credited certain specific chemical constituents of the tubercle bacillus, obtained from its lipoid fraction, with a selective affinity for and action on this cell. The monocytes entering into the early formation of the tubercle, according to their view, come in part from monocytes of the blood stream, but to a larger extent from primitive monocytes already present among the fixed elements of the infected tissues. Other investigators have traced the origin of the tubercle to tissue cells of the histiocytic or clasmotocytic class, in general to cells of the so-called "reticulo-endothelial system," which vary in appearance and development according to the exigencies of their environment. The monophyletic school of hematologists have refused to make fundamental distinctions in the nature of the reacting mononuclear cells, tracing them all back, in spite of their great morphologic variation in individual lesions, to a single stem cell (e. g., Maximow<sup>3</sup>).

The intensity of interest in the mononuclear cells entering into the formation of the epithelioid tubercle has drawn attention away from the

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1. Cunningham, R. S.; Sabin, F. R.; Sugiyama, S., and Kindwall, J. A.: *Bull. Johns Hopkins Hosp.* **37**:231, 1925.

2. Sabin, F. R.; Doan, C. A., and Forkner, C. E.: *J. Exper. Med.* (supp. 3) **52**:1, 1930.

3. Maximow, A. A.: *J. Infect. Dis.* **34**:549, 1924.

cell long known to be the first responding element on infection with tubercle bacilli, but not considered of much significance in tubercle formation, viz., the neutrophilic polymorphonuclear leukocyte. One of us (Dr. Vorwald;<sup>4</sup> see his paper for literature on the subject) recently emphasized this earliest response as of genuine importance in the development of the tubercle. He stressed the fact that investigations on the "early" formation of the tubercle, dealing with its development in the first few days, have largely overlooked the truly early changes occurring in the first few hours. In a study of tubercle formation in the rabbit's lung after intravenous infection with the human type of tubercle bacilli, he showed that an initial intense intracapillary leukocytic response occurs, which is spectacular at one hour. Even at this early period, it is difficult to find a tubercle bacillus not taken up by a polymorphonuclear leukocyte. His results indicate clearly that cells of this group in a highly effective way concentrate into small sharply localized cellular masses the bacilli introduced into the general blood stream. These masses are the groundwork on which the tubercle develops. The polymorphonuclear response under the conditions of this experiment thus definitely determines the location of development of the future tubercles. As the hours go by (the process is particularly well seen at the period from the fourteenth to the eighteenth hour), large mononuclear exudate cells gradually infiltrate the polymorphonuclear mass, phagocytose the cells of this mass, and at the same time take over into their own cytoplasm the tubercle bacilli previously ingested by the neutrophilic leukocytes. An almost complete replacement thus takes place, so that at twenty-four hours a tubercle is seen that is predominantly mononuclear, with the tubercle bacilli almost exclusively in cells of this class. Subsequent modifications of these cells, by the nuclear and cytoplasmic changes that Cunningham, Sabin and their co-workers<sup>1</sup> and Sabin, Doan and Forkner<sup>2</sup> described, convert them into typical epithelioid cells.

The results just described occur on primary infection. It is well known that important differences from the characteristics of primary infection occur when tubercle bacilli are introduced into the tissues of animals already infected with tuberculosis. It is likewise true that previous treatment with dead tubercle bacilli modifies the course of subsequent infection with virulent tubercle bacilli. Animals thus treated not only react positively to tuberculin, but possess a considerable degree of immunity.<sup>5</sup> Moreover, in animals immunized by treatment with dead bacteria, in addition to the general immunity, an exceptionally high local

4. Vorwald, A. J.: *Am. Rev. Tuberc.*, to be published.

5. Petroff, S. A.: *Am. Rev. Tuberc.* 7:412, 1923. Lange, B.; Freund, R., and Jochimsen, E.: *Ztschr. f. Hyg. u. Infektionskr.* 109:426, 1927.

immunity has been demonstrated. This was especially well shown by Gay and his associates<sup>6</sup> for streptococci in the pleural cavity and by Cannon and Pacheco<sup>7</sup> for staphylococci in the skin. In view of all these facts it seemed desirable to supplement Vorwald's experiments on primary infection with a study of the reaction at early hours in animals the reacting capacity of which had been modified by previous infection and artificial immunization.

For a number of reasons, the testis of the guinea-pig was chosen for this experiment. In the first place, it probably exhibits the allergic reaction of reinfection with a higher degree of intensity than any other organ of the body.<sup>8</sup> Parenchymatous degenerative changes and interstitial inflammatory changes are both profound. Secondly, the external location and isolated character of the testis render it especially suitable for an experiment in which exact and equal dosage in a series of animals is desirable. Finally, the choice of this organ made possible a comparison of the effects of general and local immunization on the development and course of a subsequently induced local tuberculosis. For this phase of the experiment, one testis was locally immunized by several injections of dead tubercle bacilli. Animals of this group thus could be used for a comparative study of the effects of local and general immunization in the same animal, the reaction to live tubercle bacilli in the immunized testis serving as an index of the effect of local immunization, and the reaction to an equal dosage of bacilli in the other testis, as an index of the degree and character of general immunization.

#### PLAN OF EXPERIMENT

Three groups of guinea-pigs were used: (1) normal guinea-pigs, (2) guinea-pigs rendered tuberculous by an injection of virulent human type tubercle bacilli (Saranac Lake H<sub>37</sub>) one month previously into the left axilla, and hypersensitive to tuberculin at the time of the experiment, as proved by cutaneous tuberculin tests, and (3) guinea-pigs that had been treated by five injections of 0.1 mg. of a suspension of heat-killed human type tubercle bacilli (H<sub>37</sub>) at intervals of two, four, two and four days and that had been allowed to rest one month after the fifth injection of the dead bacilli. Several facts of interest developed in connection with this immunizing procedure. As was anticipated from the work of Gay and his associates<sup>6</sup> and that of Cannon and Pacheco,<sup>7</sup> extensive scarring resulted, with the production of a moderately vascular granulation tissue extensively infiltrated by cells of inflammation, especially lymphocytes and large mononuclear exudate cells. The seminiferous tubules were not wholly destroyed by the procedure, many persisting with characteristic spermatogenesis.

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Infecting injections were then made as follows: 0.1 mg. of H<sub>37</sub> tubercle bacilli per kilogram of animal weight (about 0.05 mg. per animal) was injected into the left testis in group 1, the left testis in group 2 and both testes in group 3. Animals of each group were then killed at the end of one, three, six, ten, sixteen, twenty-four, forty-eight and seventy-two hours. The testes into which the injections had been made were fixed in Zenker's fluid plus formaldehyde and stained with hematoxylin and eosin for general observation, eosin-azure for particular study of the leukocytes, and fuchsin, hematoxylin and orange-G for study of the tubercle bacilli.

#### OBSERVATIONS: AT ONE HOUR

*Normal Guinea-Pig, Left Testis.*—Some bacilli were found clumped outside of cells; occasionally polymorphonuclear leukocytes were in their neighborhood, and a few bacilli were within polymorphonuclears. The blood vessels contained an excess of polymorphonuclears. Tubercle bacilli were not seen in mononuclear cells. In general, little reaction was present; on superficial examination, the sections would be taken for those of a normal testis.

*Tuberculous Guinea-Pig, Left Testis.*—Bacilli in moderate number were clumped in groups of polymorphonuclear leukocytes and in clumps outside. Allergic reaction, as manifested by an abnormal degree of exudation and degeneration of parenchymatous cells, was not yet apparent. In general, the testis looked like the left testis of the normal guinea-pig.

*Immunized Guinea-Pig, Left Testis (Locally Immunized).*—The testis was small, and its tubules were atrophied; in general, it consisted of scar tissue infiltrated by cells of inflammation of all types, fibroblasts predominating (the effect of local immunization). In one area there was a region of suppuration. Tubercle bacilli were seen, occasionally extracellular, occasionally in polymorphonuclears and occasionally in large mononuclears.

*Immunized Guinea-Pig, Right Testis.*—Bacilli were found outside of cells, without appreciable reaction; others were in the midst of a group of polymorphonuclear leukocytes. In each case, they were clumped. The testis was otherwise unchanged.

*Summary.*—The reaction at this period was slight in every case. No striking difference was seen in the different groups. A tendency to clumping of the bacilli was seen in all testes. What little reaction there was was chiefly on the part of polymorphonuclear leukocytes, which had concentrated the bacilli in groups and accomplished some phagocytosis. In the locally immunized testis, where numerous large mononuclears were already present, some phagocytosis by this type of cell had occurred, but it was not conspicuous.

#### AT THREE HOURS

*Normal Guinea-Pig, Left Testis.*—Clumps of bacilli were found in masses of polymorphonuclear leukocytes, approximately 15 to 20 leukocytes per group in a single section plane. These groups were concentrated in the center of the organ.

*Tuberculous Guinea-Pig, Left Testis.*—The testis was hyperemic and definitely the seat of an early tuberculin reaction, with barely recognizable degeneration of germ cells, but moderately extensive, diffuse intertubular infiltration by cells of inflammation. These were chiefly polymorphonuclear leukocytes and eosinophils. Tubercle bacilli were found in polymorphonuclear leukocytes and not extracellularly.

*Immunized Guinea-Pig, Left Testis (Locally Immunized).*—The testis was much scarred and contained cells of inflammation of all sorts, including many eosinophils. Spermatogenesis was not entirely destroyed. Tubercle bacilli were concentrated in accumulations of polymorphonuclear leukocytes and not in the large mononuclear cells present.

*Immunized Guinea-Pig, Right Testis.*—Near the center of the organ was a region of accumulation of polymorphonuclear leukocytes. Among these leukocytes were many clumps of tubercle bacilli. The region of accumulation had a diameter equal to that of two tubules.

*Summary.*—The reacting cell was the polymorphonuclear leukocyte in all instances, including the locally immunized testis already infiltrated by large mononuclears with phagocytic capacity. The testis of the tuberculous animal, even at this early stage, showed evidence of its general hypersensitiveness in the extent and diffuseness of the intertubular infiltration and the slight degenerative changes in the germ cells.

#### AT SIX HOURS

*Normal Guinea-Pig, Left Testis.*—There was no appreciable tubular degeneration. Small accumulations of polymorphonuclear leukocytes containing tubercle bacilli were now present between the tubules. There were similar accumulations between tubules, without tubercle bacilli.

*Tuberculous Guinea-Pig, Left Testis.*—The tubules were swollen and the germ cells degenerated. There was an intense exudation of cells of inflammation and fibrin between the tubules. The reaction was more diffuse than in the immunized guinea-pig. The cells of the general exudate were chiefly polymorphonuclears, but a moderate number of mononuclears and a moderate number of eosinophils were also present. Here and there were small accumulations of polymorphonuclears, resembling small abscesses, in which tubercle bacilli were clumped.

*Immunized Guinea-Pig, Left Testis (Locally Immunized).*—The testis was much scarred and infiltrated by cells of inflammation of all sorts; eosinophilic leukocytes were especially conspicuous. At one margin of the testis, there was a large old abscess with a necrotic center surrounded by polymorphonuclears. Scattered throughout the testis were small intertubular abscesses made up of polymorphonuclear leukocytes containing tubercle bacilli. The mononuclears, which were so prominent a part of the picture (as a result of the local immunization), did not take part in the reaction to the tubercle bacilli. No phagocytosis by eosinophils was seen, although these cells were present in enormous number.

*Immunized Guinea-Pig, Right Testis.*—The tubules were normal, but throughout the testis there was an intertubular infiltration by cells of inflammation, among which eosinophils were extremely numerous. Small, dense accumulations of polymorphonuclear leukocytes containing tubercle bacilli were between the tubules. Eosinophils were extremely numerous in the blood vessels. The cells immediately around the bacilli were almost exclusively polymorphonuclear neutrophils. Aside from the scarring previously produced in the locally immunized testis, the reaction was the same in the right and the left testis of this guinea-pig.

*Summary.*—A difference in the degree and character of the reaction in the different groups was now readily apparent. In the normal guinea-pig, the reaction was slight and chiefly polymorphonuclear. In the tuberculous guinea-pig, an intense inflammatory reaction had developed, and in further contrast to the normal animal, the parenchymatous cells showed beginning degeneration (a well recognized feature of the tuberculin reaction in the testis). A feature specifically characteristic in

this animal was the exudation of fibrin. In the animal immunized by injection of dead bacilli, the intensity of the cellular response lay between that of the normal and that of the tuberculous animal. The difference from the normal indicated a distinct allergic element. Absence of tubular degeneration in the right testis of the immunized animal showed that vaccination of the animal with dead bacilli had not brought about as intense sensitization of the parenchymatous cells as actual tuberculosis would have. A noteworthy feature of the allergic reaction was the exudation of eosinophilic leukocytes, which, in contradistinction to the neutrophils, carried out no phagocytosis. The actively reacting cell in all groups was the polymorphonuclear neutrophil. This was true of the locally immunized testis, in spite of the fact that it was full of mononuclears before the injection of tubercle bacilli (fig. 1 *A* and *B*; fig. 2 *A* and *B*).

#### AT TEN HOURS

*Normal Guinea-Pig, Left Testis.*—There was no appreciable tubular degeneration. There was definite but only moderate intertubular infiltration by cells of inflammation. Most of these were polymorphonuclear leukocytes, but many plasma cells and some lymphocytes and eosinophils were also present. Eosinophils were fewer than in the immunized animals. Tubercle bacilli were found in clumps in and among polymorphonuclear leukocytes.

*Tuberculous Guinea-Pig, Left Testis.*—Polymorphonuclear leukocytes were extremely numerous in the small blood vessels. In some vessels, these cells were accumulated to such a degree as to produce the picture of small septic thrombi (fig. 3). No bacilli were found in these intravascular leukocytes. Otherwise, the picture resembled that at six hours.

*Immunized Guinea-Pig, Left Testis (Locally Immunized).*—This was a typical immunized testis, uniformly scarred with connective tissue and infiltrated by lymphocytes and large mononuclear exudate cells. Bacilli were found in polymorphonuclear leukocytes, which were present in small scattered masses, although in this instance only in small numbers.

*Immunized Guinea-Pig, Right Testis.*—The tubules showed very slight degeneration. On the whole, the picture resembled that at six hours, except that there was a larger accumulation of polymorphonuclear leukocytes with tubercle bacilli near the center of the organ.

*Summary.*—The picture differed from that at six hours chiefly in that, in general, the reactions were more intense. Parenchymatous degeneration was now becoming apparent in the right testis of the guinea-pig immunized with dead bacilli, an effect not yet noticeable in the normal animal, evidence that vaccination with dead bacilli sensitized these cells to some extent. The degree of this degeneration was much greater in the tuberculous animal than in the animal immunized with dead bacilli. The polymorphonuclear leukocytic response was still the dominant reaction, and its intensity in the tuberculous and immunized animals greatly surpassed its intensity in the normal.

#### AT SIXTEEN HOURS

*Normal Guinea-Pig, Left Testis.*—Tubular degeneration was hardly appreciable, but there was a great increase in intertubular infiltration by cells of inflammation, and in these intertubular infiltrations were small definite abscesses consisting of polymorphonuclear leukocytes with tubercle bacilli.



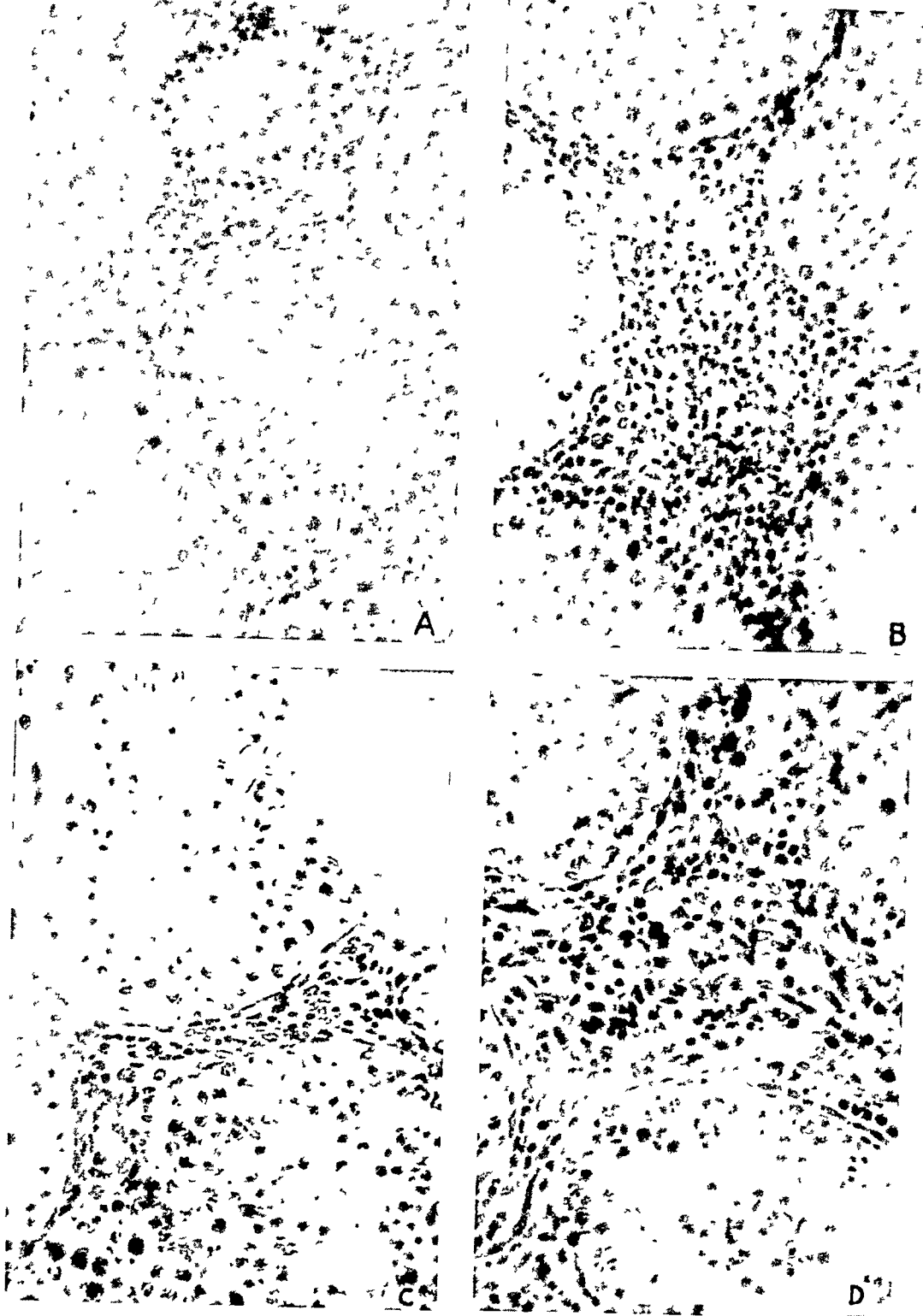


Fig. 1.—*A*, the left testis of a normal guinea-pig six hours after an injection of virulent tubercle bacilli. Note the margination of leukocytes in the blood vessel and the slight exudate between the seminiferous tubules. *B*, the left testis of a tuberculous guinea-pig six hours after an injection of virulent tubercle bacilli. Note the large size of the intertubular exudate in comparison with that in *A*. *C*, the left testis of a normal guinea-pig seventy-two hours after an injection of virulent tubercle bacilli. Note that the intertubular exudate now consists chiefly of mononuclear cells. *D*, the left testis of a tuberculous guinea-pig seventy-two hours after an injection of virulent tubercle bacilli. Note that the intertubular exudate is much larger than in *C* and approximately equal in size to that in *B*; it now consists chiefly of mononuclear cells. Note the degeneration of the seminiferous tubules.

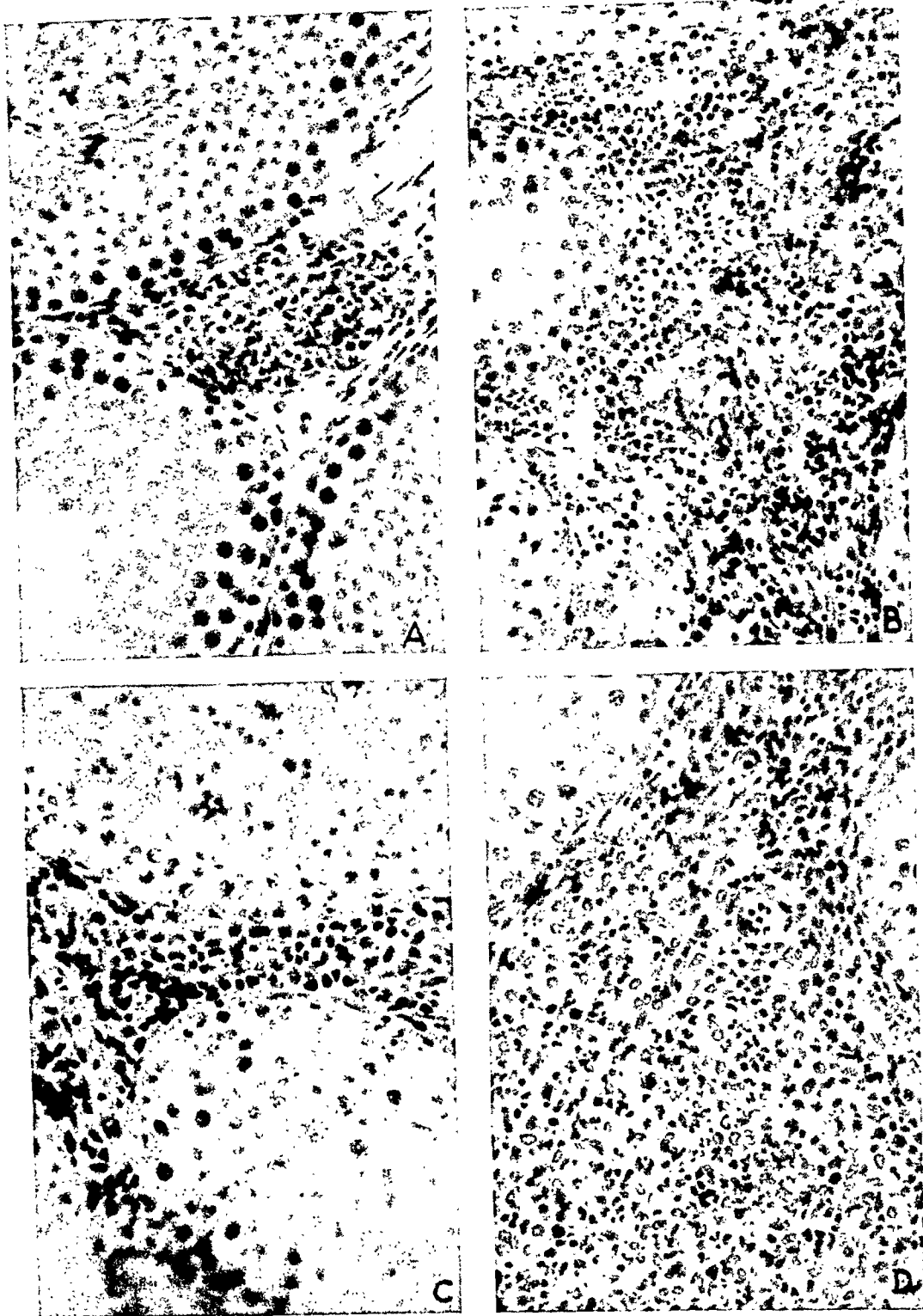


Fig. 2.—*A*, the right testis of a guinea-pig immunized by repeated injections of dead tubercle bacilli into the left testis, six hours after an injection of virulent tubercle bacilli. Note the exudation of polymorphonuclear leukocytes between tubules. The size of the region of exudation is between that seen in figure 1 *A* and *B*. *B*, the left testis from the same animal as *A*, also six hours after an injection of virulent tubercle bacilli. Note the extensive mononuclear leukocyte infiltration and the tubular degeneration due to local immunization with dead bacilli. Note also the extensive infiltration by polymorphonuclear leukocytes due to the recent injection of live tubercle bacilli. *C*, the right testis of a guinea-pig immunized by repeated injections of dead tubercle bacilli into the left testis, seventy-two hours after an injection of virulent tubercle bacilli. Compare with *A*, and note that the exudate now consists chiefly of mononuclear leukocytes. *D*, the left testis from the same animal as *C*, also seventy-two hours after an injection of virulent tubercle bacilli. The intertubular exudate consists almost entirely of mononuclear cells, the polymorphonuclear leukocytes characteristic of the reaction in *B* having been replaced.

*Tuberculous Guinea-Pig, Left Testis.*—The reaction was a typical one of reinfection, but happened in this case to be less marked than at ten hours. Tubercle bacilli were seen in polymorphonuclear leukocytic abscesses as usual. Large mononuclear leukocytes were beginning to mingle with polymorphonuclears in these abscesses.

*Immunized Guinea-Pig, Left Testis (Locally Immunized).*—The reaction was essentially the same as previously described for the comparable testis in the ten hour animal.

*Immunized Guinea-Pig, Right Testis.*—Tubular degeneration was now definite, but less intense than in the tuberculous animal. Intertubular infiltration was increasing greatly, with many dense accumulations of polymorphonuclears containing tubercle bacilli.

*Summary.*—The picture was much like that at ten hours.



Fig. 3.—A blood vessel in a zone of inflammatory edema in the testis of a tuberculous guinea-pig, packed with polymorphonuclear leukocytes ten hours after an injection of virulent tubercle bacilli. This powerful chemotaxis for leukocytes is one manifestation of a tuberculin reaction.

#### AT TWENTY-FOUR HOURS

*Normal Guinea-Pig, Left Testis.*—Tubular degeneration was now beginning to be noticeable. Intertubular infiltration by cells of inflammation was like that at sixteen hours, but more concentrated in spots. Tubercle bacilli were present in the accumulations of polymorphonuclear leukocytes.

*Tuberculous Guinea-Pig, Left Testis.*—A tuberculin reaction of profound severity was present, with intense degeneration of the tubular cells and exudation of cells of inflammation and fibrin. Tubercle bacilli were still found, for the most part, in polymorphonuclears; nevertheless, large mononuclears were beginning to appear in these regions, among the polymorphonuclear leukocytes.

*Immunized Guinea-Pig, Left Testis (Locally Immunized).*—Less scarring than usual had resulted from the local immunization. Small polymorphonuclear abscesses with bacilli in and among the leukocytes were still found in the regions of lymphocytic and mononuclear infiltration due to the immunizing.

*Immunized Guinea-Pig, Right Testis.*—Tubular degeneration was apparent, but not marked. Otherwise, the picture was the same as in the corresponding testis at sixteen hours.

*Summary.*—The reaction of hypersensitiveness in the testis of the tuberculous guinea-pig was now at its height; its chief features were intense intertubular exudation, in which fibrin and leukocytes were abundant, and profound tubular degeneration. The right testis of the animal immunized by injections of dead bacilli into the left testis was the seat of a moderately diffuse intertubular exudate in which fibrin was less conspicuous; tubular degeneration had occurred, but was only moderate. In the left testis, locally immunized, the reaction to the recently introduced bacilli was still polymorphonuclear leukocytic. In the previously normal guinea-pig the reaction was less intense than in any of the others, and was less diffuse; twenty-one hours later than in the tuberculous guinea-pig parenchymatous degeneration was beginning to be seen.

#### AT FORTY-EIGHT HOURS

*Normal Guinea-Pig, Left Testis.*—Tubular degeneration was now definite, although slight as compared with that in a tuberculin reaction. Intertubular cellular infiltration had increased, but the cells were now predominantly large mononuclears. Definite concentrations of polymorphonuclear leukocytes, equivalent to abscesses, were present and full of tubercle bacilli. The number of bacilli in these accumulations was extraordinary; the colony-like arrangement, as well as the number of bacilli present, was such as to suggest actual bacillary multiplication. The bacilli were unusually granular.

*Tuberculous Guinea-Pig, Left Testis.*—This testis had a tubercle in it (hematogenous from the original infection). Outside of this, it showed a profound tuberculin reaction. Regions of polymorphonuclear accumulation and others made up chiefly of mononuclears were present.

*Tuberculous Guinea-Pig, Right Testis* (An injection was made into the right testis because of the condition of the left).—There was a typical tuberculin reaction, though tubular damage was not so profound. The cells reacting to the tubercle bacilli were now about 50 per cent polymorphonuclears and 50 per cent large mononuclears. A conspicuous feature was the phagocytosis by large mononuclear leukocytes of polymorphonuclears containing tubercle bacilli.

*Immunized Guinea-Pig, Left Testis (Locally Immunized).*—The testis was much scarred, with large areas of lymphocytic infiltration (from the immunizing procedure). The lymphatics in this region were packed with lymphocytes (local production of lymphocytes). Throughout the region of lymphocytes were accumulations of polymorphonuclears, virtually small abscesses in the midst of the regions of lymphocytic infiltration; in them, tubercle bacilli were numerous. Most of the bacilli were in polymorphonuclears, but some were in large mononuclears. The mononuclears now made up about 25 per cent of the cellular infiltration. The polymorphonuclear leukocytes were disintegrating, and there was a good deal of phagocytosis of debris, tubercle bacilli and polymorphonuclears by the large mononuclears.

*Immunized Guinea-Pig, Right Testis.*—Tubular degeneration was conspicuous and intertubular infiltration greatly increased. From 50 to 75 per cent of the reacting cells were polymorphonuclear leukocytes and eosinophils; 25 per cent or more were mononuclears. The bacilli were now found especially in the large mononuclears.

*Summary.*—The chief change that had occurred was the infiltration by large mononuclear leukocytes of the foci of polymorphonuclear leukocyte concentration. The mononuclears were actively phagocytosing the polymorphonuclears with their bacillary content. The mononuclear reaction was of approximately equal proportionate intensity in all groups of animals. It was no more conspicuous in the locally immunized testis than in the others. The intensity of total reaction in the several groups varied in the same manner as at previous hours.

#### AT SEVENTY-TWO HOURS

*Normal Guinea-Pig, Left Testis.*—Tubular damage was apparent, although still very slight as compared with that in a tuberculin reaction. The regions of cellular infiltration had distinctly decreased in size; they were now scattered and compact and the cells present were chiefly mononuclears, and there was much phagocytosis of bacilli by mononuclears; phagocytosis was now practically confined to this type of cell.

*Tuberculous Guinea-Pig, Left Testis.*—The picture was that of a typical advanced tuberculin reaction. There was extreme degeneration of the tubules. Interstitial infiltration by cells of inflammation was extensive; these were now in a large majority mononuclears. Much fibrin was still present. Polymorphonuclears were few; they were seen occasionally phagocytosed by mononuclears. Mitotic figures in the exudate cells were occasionally seen. Bacilli were hard to find, but when present were seen in large mononuclear cells.

*Immunized Guinea-Pig, Left Testis (Locally Immunized).*—The typical picture of immunization scarring was present. Polymorphonuclear abscesses had practically disappeared. Remains of such abscesses could be distinguished, heavily infiltrated with large mononuclears. Polymorphonuclears were few. Tubercle bacilli were in the mononuclears, and the bacilli were smaller and more faintly staining.

*Immunized Guinea-Pig, Right Testis.*—The picture was that of a tuberculin reaction. There was marked degeneration of tubules, with intense interstitial infiltration; the cells of the latter, however, were almost exclusively mononuclears. The infiltration, while diffuse throughout the testis, was much more compact than before; where formerly it had been a collection of cells, now it began to resemble a tissue. Polymorphonuclears were very few. Bacilli were found in the mononuclears. Phagocytosis of polymorphonuclears containing bacilli by mononuclears could be seen. These bacilli were small, thin and pale staining.

*Summary.*—Throughout the series, the polymorphonuclear leukocytic response, which was so prominent at periods up to twenty-four hours, had practically disappeared. In all cases, large mononuclear exudate cells had invaded the leukocytic accumulations, phagocytosing the polymorphonuclears and taking over their content of bacilli. This change had brought about a general reduction in the size of the reacting zones. This was accompanied by a developing compactness, which gave to the zones of inflammatory reaction the character of a tissue, rather than of a collection of exuded cells. Degeneration of tubular epithelium was apparent in all cases, most intense in the tuberculous animal, least intense in the normal and of medium intensity in the animal immunized by previous injection of dead bacilli (fig. 1, C and D; fig. 2, C and D).

Figures 1 and 2 illustrate the difference in reaction in the four groups at six and seventy-two hours. In figure 1, the reactions in the normal and tuberculous animals at six and seventy-two hours are compared. In figure 2, the same comparison is made for the locally immunized testis and the opposite testis of the same animal.

#### COMMENT

A distinct positive correlation is apparent between the intensity of inflammatory reaction in these experiments and the presumable degree of immunity of the animals. Present-day researches indicate that guinea-pigs immunized with dead tubercle bacilli resist invasion of live bacilli better than normal animals, while animals with mild actual tuberculosis resist new infection still more strongly than animals immunized with dead bacilli. The range of immunity to fresh tuberculous infection is thus as follows: least in normal guinea-pigs; intermediate in guinea-pigs immunized with dead bacilli; greatest in guinea-pigs with mild active tuberculosis. In the experiments here recorded, the intensity of inflammatory reaction to freshly introduced tubercle bacilli varied in the same order; that is, it was least in normal animals, of intermediate grade in guinea-pigs immunized with dead bacilli and greatest in those with already present tuberculosis.

The correlation is so striking as to appear more than a coincidence, and it would be easy to assume that the more resistant state of the immunized and tuberculous animals was directly due to the intensity of cellular reaction brought to bear on the freshly introduced bacilli. Certainly this reaction brought about a localization of bacilli in small discrete foci in all animals studied. Nevertheless, as studies by Vorwald (in press) indicate, intensity of inflammatory reaction in tuberculosis cannot always be correlated directly with resistance. In a series of animals of greatly varying native immunity, such a correlation did not exist. Therefore, the results here recorded are published simply to furnish a quantitative comparison of the early inflammatory reaction at successive time periods in the four states of reactive capacity studied. Certain other factors are still to be evaluated before the exact relation of this variation in reaction intensity to actual immunity is known.

#### SUMMARY

Virulent tubercle bacilli (Saranac Lake strain H<sub>37</sub>), in a dosage of 0.1 mg. per kilogram of animal weight, were injected into the testes of normal and tuberculous guinea-pigs and guinea-pigs previously treated by repeated intratesticular inoculation of a suspension of dead tubercle bacilli. The last named group of animals served a double purpose, in that inoculation of live bacilli into the testis into which dead bacilli had been previously injected elicited a response in a tissue that might be considered locally immunized, while injection in the opposite nontreated

testis elicited a response presumably characteristic of a general immunized state of the animal. The testis was chosen because the reactions of hypersensitiveness in this organ in the guinea-pig are intense.

The cellular reactions in these four states of testis tissue (normal testis of the normal animal, normal-appearing testis of the tuberculous animal, scarred testis of the locally immunized animal and the normal-appearing opposite testis of the same animal) were examined one, three, six, ten, sixteen, twenty-four, forty-eight and seventy-two hours after the inoculation of live virulent bacilli. In all cases, the most actively reacting cell at the early hours was the polymorphonuclear leukocyte. Phagocytosis of bacilli by these cells was practically complete in three hours. The most intense exudation of these cells at the early periods occurred in the testes of the tuberculous animals. In this group, a great deal of fibrin appeared in the exudate, and at the same time a rapid degeneration of the seminiferous tubules occurred. (This combination of changes is characteristic of the tuberculin reaction in the testis, and is due, as previous studies have shown, to the protein of the tubercle bacillus.) The next most intense exudation of polymorphonuclear leukocytes occurred in the guinea-pigs treated with repeated injections of dead tubercle bacilli. The reaction was of approximately equal intensity in the locally immunized testis and the opposite testis modified in reactive capacity only by the general immunization conferred by the local immunizing procedure. Special attention is called to the condition of the locally immunized testis. Although this testis was full of mononuclear cells of inflammation as a result of its repeated inoculation with dead bacilli, the early reaction to freshly introduced live bacilli was a polymorphonuclear one, just as in all other animals. The least intense exudative reaction occurred in the testes of the normal animals.

Between twenty-four and forty-eight hours after the inoculation with live bacilli, a gradual replacement of the polymorphonuclear leukocytes by large mononuclear exudate cells occurred in all animals. Extensive phagocytosis of polymorphonuclear leukocytes by large mononuclears took place. In this way, a transference of tubercle bacilli from the cytoplasm of the polymorphonuclears to that of the large mononuclears was accomplished. This rôle of the large mononuclear leukocytes did not appear earlier in the locally immunized testes than in the other testes of the series. At seventy-two hours, the familiar picture of scattered early mononuclear cell tubercles was present, each of these tubercles representing, it should be noted, a previous site of focalization of polymorphonuclear leukocytes and bacilli. Maturation of these large mononuclears to the typical epithelioid appearance had not yet taken place. (Other studies have shown that, on a large scale, this takes place in about two weeks.)

## CONCLUSIONS

It may be concluded from these experiments that prompt localization of tubercle bacilli is brought about by exudation of polymorphonuclear leukocytes and phagocytosis of the bacilli by these cells. Although the bacilli are at first widely spread and the initial polymorphonuclear reaction is correspondingly diffuse, aggregation of the leukocytes, containing all the bacilli injected, into distinct and separate foci, soon occurs. The site of future true tubercle formation is thus determined, for the polymorphonuclears are soon replaced by large mononuclears, which, as shown by the experience of many other investigators, later develop into epithelioid cells with more abundant cytoplasm. The fact that replacement of the polymorphonuclear mass by large mononuclears did not occur appreciably earlier in the locally immunized testis, already full of mononuclear cells, than in the other testes of the series suggests that in these experiments, at least, the large mononuclear cells concerned in the reaction came directly from the blood stream and were not derived locally. An already present tuberculosis in the animal greatly accelerates the reacting capacity of the polymorphonuclear leukocytes. This may be looked on as simply one feature of a typical tuberculin reaction. Previous inoculation with dead bacilli brings about a similar allergic state, although the acceleration and increased intensity of inflammatory reaction over that seen in the normal animal are not nearly so great as in the truly tuberculous animal.



# General Reviews

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## PELLAGRA

ETIOLOGY (MODERN THEORIES) AND PATHOLOGIC ANATOMY \*

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Pellagra has been related to warm climates since its discovery in Spain by Casal<sup>1</sup> in 1735. The description of the disease in France during the first part of the last century by Hameau<sup>2</sup> places it in the southwestern part of that country. The prevalence of pellagra in Italy early in the history of the disease, reported in the important monographs of Frapolli,<sup>3</sup> Zanetti,<sup>4</sup> Strambio,<sup>5</sup> Gherardini<sup>6</sup> and others, associated the disease with climatic conditions. The invasion of the United States by pellagra during the past two decades has been confined, as is generally known, mainly to the southern states.

Maize was observed to be the principal article in the food of pellagrins in the lower latitudes. The maize theory of the etiology of pellagra was advanced for many years, from which was evolved the deficiency theory. The relation of sunlight to the disease brought forth the photodynamic theory. The investigations of micro-organisms as a cause of other infections naturally introduced the parasitic theory. Research has been directed mainly from these hypotheses during the last twenty years.

### THE DEFICIENCY THEORY

The importance of maize in the diet gave rise to the zeists, whose ideas flourished for many decades. During the last century, the maize theory was advanced by such prominent workers as Marzari,<sup>7</sup> Balardini,<sup>8</sup>

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\* Submitted for publication, March 28, 1931.

\* From the Department of Pathology, University of Arkansas, School of Medicine.

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Morelli<sup>9</sup> and Roussel.<sup>10</sup> This theory was regarded as defective when it became known that pellagra exists in countries in which Indian corn is not consumed. The extensive investigations of the Illinois commission<sup>11</sup> struck another blow to zeism, because it could not relate maize as an etiologic factor.

During the present century extensive search has been made to discover the exact factor that is missing in the diet. Outbreaks of the disease have occurred in which faulty diets were proved. Studies were made of the diets of pellagrins among Armenian refugees<sup>12</sup> and Turkish and German prisoners<sup>13</sup> at the time of the World War, but the exact deficiency remained somewhat uncertain. Enright,<sup>14</sup> after a critical analysis of the diets of German prisoners, did not believe that a food deficiency was related to this disease. MacNeal<sup>15</sup> noted that no outbreaks had occurred in France, England, Germany or Austria during the war. I saw hundreds of impoverished French refugees with no signs of pellagra. At that time dietary studies on protein food values were considered, and a theory developed that pellagra was caused by a deficiency of protein in the diet. Goldberger,<sup>16</sup> Voegtlin,<sup>17</sup> Goldberger, Waring and Willets<sup>18</sup> and Wheeler<sup>19</sup> gave additional proofs of the prevention or of the cure of the disease. Jobling and Peterson<sup>20</sup> in their extensive studies on the epidemic in Nashville noted two factors that were contradictory: A certain number of cases occurred when the diet was wholesome; at least half of the cases developed where there was a monotonous diet low in protein and high in carbohydrates. Although the protein deficiency theory was advanced, it was not established.

The late pellagrologist, Joseph Goldberger,<sup>21</sup> emphasized with considerable force his view that a faulty supply of protein (amino-acid)

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18. Goldberger, J.; Waring, C. H., and Willets, D. G.: *Pub. Health Rep.* **29**:2821, 1914.

19. Wheeler, G. A.: *Pub. Health Rep.* **39**:2197, 1924.

20. Jobling, J. W., and Peterson, W.: *J. Infect. Dis.* **18**:501, 1916; **21**:109, 1917.

21. Goldberger, J.: *J. A. M. A.* **66**:471, 1916; **78**:1676, 1922; **80**:1866, 1923. Goldberger, J., and others: *Pub. Health Rep.* **41**:297, 1926.

is the primary cause of pellagra. Prior to Goldberger's extensive publications the Illinois commission<sup>11</sup> noted that pellagra had decreased in certain hospitals with an increase in the consumption of meat. Goldberger's work was conscientiously planned and performed, but his conclusions might be questioned. The dermatitis that he produced in six convicts on a diet deficient in protein has been questioned. Lesions of the skin appeared first on the scrotum in six convicts and on the backs of the hands in two. This dermatitis has not been accepted as diagnostic of pellagra by all of the critics. MacNeal<sup>15</sup> and Hindhede<sup>22</sup> were probably correct when they considered Goldberger's<sup>21</sup> conclusions somewhat uncertain. The most important criticism that can be offered to this experiment on the prison farm, one which was mentioned by Jobling and Peterson,<sup>20</sup> is that it was not accomplished in a nonpellagrous community, even if it were demonstrated that the convicts develop unquestionable pellagrous symptoms. The experimental lesions produced in monkeys by Chick and Hume<sup>23</sup> on a diet low in protein could not be called pellagrous without question.

Funk<sup>24</sup> advanced the idea that pellagra might be caused by lack of a vitamin. Vedder<sup>25</sup> believed that there was resemblance between pellagra, scurvy and beriberi and compared the changes in the nervous system to those in beriberi. Since the discovery that vitamin B complex is composed of two factors, deficiency of vitamin G<sup>26</sup> has been considered to be the cause of pellagra. There was lack of vitamin G in the diet of pellagrins whom I<sup>27</sup> observed in an outbreak after the inundation in Arkansas in 1927. Irby,<sup>28</sup> who studied the diets of pellagrins in Arkansas after the drought, found a deficiency of vitamin G. Goldberger<sup>29</sup> reported the presence of vitamin G in various foods especially in yeast and in lean meat. Aykroyd and Roscoe<sup>30</sup> found that dried ox liver, yeast and fresh whole milk were excellent sources of vitamin B<sub>2</sub>. Since meat, milk and especially yeast were important articles required for prevention or cure, it was natural to relate vitamin G as the necessary pellagra-preventing factor. Vitamin G caused pellagra-like symptoms in the albino rat to disappear, and this vitamin

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22. Hindhede, M.: *J. A. M. A.* **80**:1685, 1923.

23. Chick, H., and Hume, M.: *Biochem. J.* **14**:135, 1920.

24. Funk, C.: *J. Physiol.* **47**:389, 1913. Eddy, W. H.: *Vitamin Manual*, Baltimore, Williams & Wilkins Company, 1921.

25. Vedder, E. B.: *Arch. Int. Med.* **18**:137, 1916.

26. The letter "G" has been adopted by the Committee on Nomenclature appointed by the American Society of Biological Chemists to indicate the stable factor associated with the vitamin B complex. The British biochemists have provisionally adopted the term "B<sub>2</sub>."

27. Thatcher, H. S.: *J. Arkansas M. Soc.* **24**:193, 1928.

28. Irby, Patricia: Personal communication to the author, 1930.

29. Goldberger, J.: *Pub. Health Rep.* **42**:2193, 1927.

30. Aykroyd, W. R., and Roscoe, M. H.: *Biochem. J.* **23**:483, 1929.

was then designated as the antipellagric vitamin. Goldberger now changed his opinion to comply with the vitamin G deficiency theory.

Experimental production of pellagra-like symptoms in the albino rat has been reported by Goldberger and Lillie,<sup>31</sup> Chick and Roscoe,<sup>32</sup> Hunt,<sup>33</sup> Salmon, Hays and Guerrant,<sup>34</sup> Sherman and Sandels,<sup>35</sup> Findlay,<sup>36</sup> and Thatcher, Sure and Walker.<sup>37</sup> The last mentioned workers completed a critical study with histologic examinations, but could not relate the disease absolutely to human pellagra, although symmetry of the lesions and seasonal variation were observed. Recently it was suggested by Sure,<sup>38</sup> Salmon<sup>39</sup> and Hunt and Wilder<sup>40</sup> that vitamin G consists of two factors. Sure and Smith<sup>41</sup> concluded that the growth-producing factors associated with the antipellagric vitamin are not identical. Further work should give us more evidence about this so-called antipellagric vitamin.

The experimental "black tongue" syndrome of dogs was related to pellagra and lack of protein in the diet by Underhill and Mendel. Goldberger and Wheeler<sup>42</sup> later prevented and cured "black tongue" by autoclaved yeast. Denton<sup>43</sup> stated that the distinctive lesions of pellagra and those of "black tongue" of dogs appeared to have their origin in a failure on the part of the organism to maintain the specialized supporting tissues of epithelium in various situations.

Recently it was suggested that elements other than vitamins may have some significance. Leader<sup>44</sup> claimed that in the absence of cane sugar in the diet, pellagra did not occur in experimental rats with a diet deficient in vitamin "B complex." She noted the seasonal variation that was observed by Thatcher, Sure and Walker<sup>45</sup> on another diet. Bliss<sup>46</sup> suggested that pellagra was a nondeficiency disease. He stated

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31. Goldberger, J., and Lillie, R. D.: Pub. Health Rep. **41**:1025, 1926.

32. Chick, H., and Roscoe, M. H.: Biochem. J. **21**:698, 1927.

33. Hunt, C. H.: J. Biol. Chem. **78**:83, 1928.

34. Salmon, W. D.; Hays, I. M., and Guerrant, N. B.: J. Infect. Dis. **43**:426, 1928.

35. Sherman, H. C., and Sandels, M. R.: Proc. Soc. Exper. Biol. & Med. **26**:536, 1929.

36. Findlay, G. M.: J. Path. & Bact. **31**:353, 1928.

37. Thatcher, H. S.; Sure, B., and Walker, D. J.: South. M. J. **23**:143, 1930; Arch. Path. **11**:425, 1931.

38. Sure, B.: Personal communication to the author, 1931.

39. Salmon, W. D.: J. Chem. Educ. **7**:2336, 1930.

40. Hunt, C. H., and Wilder, W.: J. Biol. Chem. **90**:279, 1931.

41. Sure, B., and Smith, M. E.: Proc. Soc. Exper. Biol. & Med. **28**:442, 1931.

42. Goldberger, J.; Wheeler, G. A.; Lillie, R. D., and Rogers, L. M.: Pub. Health Rep. **43**:1385, 1928.

43. Denton, J.: Am. J. Path. **4**:341, 1928.

44. Leader, V. R.: Biochem. J. **24**:1172, 1930.

45. Thatcher, Sure and Walker (footnote 37, second reference).

46. Bliss, S.: Science **72**:577, 1930.

that iron was present in foods containing much vitamin G, while the usual diet of the poor farmers in the South lacked iron. He further observed that dogs fed the Chittenden-Underhill diet were restored to normal by the intravenous injection of iron. Sure, Kik and Smith,<sup>47</sup> however, found anemia in rats suffering from vitamin G deficiency accompanied by lesions of the skin comparable to those found in human pellagra, when the diet contained plenty of ferric citrate. More evidence must be produced before the views of Leader<sup>44</sup> and Bliss<sup>46</sup> can be accepted.

From the analysis of the most important research relating to this theory, it is not clear that a deficient diet is the primary cause of pellagra, although there is a dietary relationship.

#### THE PHOTODYNAMIC THEORY

Fagopyrism has been regarded as of considerable importance because the dermatitis of pellagra is initiated and accentuated by sunlight. Maize was used as a source for the photodynamic substances by Hausmann,<sup>48</sup> Lode,<sup>49</sup> Horbaczewski,<sup>50</sup> Umnus<sup>51</sup> and Raubitschek.<sup>52</sup> Lesions were produced in animals inoculated with these substances when they were exposed to the sun's rays. Jobling and Arnold<sup>53</sup> did not consider it necessary to use maize as the source of the photodynamic substances because evidence had accumulated against the maize theory. They isolated organisms from the stools of pellagrins producing fluorescent substances. Extracts of these organisms were inoculated into a series of mice. When these mice were exposed to light, edema and reddening of the ears occurred, with swelling and edema of the eyelids; also gangrene of the ears developed, if inoculation and exposure were continued daily. A fungus having photodynamic properties was found in the stools of the pellagrins, which belonged to the *Aspergillus glaucus-repens* group.

Scott, Turner and Mayerson<sup>54</sup> found no difference between normal and pellagrous serum (acute cases) to warrant the conclusion that they were not spectroscopically identical. Comparison between normal serum containing traces of hematoporphyrin and pellagrous serum revealed no trace of it in the circulating blood of pellagrins. However,

47. Sure, B.; Kik, M. C., and Smith, M. E.: Proc. Soc. Exper. Biol. & Med. **28**:498, 1931.

48. Hausmann, W.: Wien. klin. Wchnschr. **23**:1287, 1910.

49. Lode, Sitzung: Wien. klin. Wchnschr. **23**:1160, 1910.

50. Horbaczewski, J.: Centralbl. f. Bakteriöl. **58**:317, 1911.

51. Umnus, O.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **13**:461, 1912.

52. Raubitschek, H.: Wien. klin. Wchnschr. **23**:963, 1910.

53. Jobling, J., and Arnold, L.: J. A. M. A. **80**:365, 1923.

54. Scott, L. C.; Turner, R. H., and Mayerson, H. S.: Proc. Soc. Exper. Biol. & Med. **27**:27, 1929.

Meyer-Betz<sup>55</sup> sensitized himself to hematoporphyrin and got edema and erythema of the face, neck and hand when exposed to the sunlight. He remained hypersensitive for six weeks, although a test for hematoporphyrin showed none present after seventy-two hours. The negative results of Scott, Turner and Mayerson<sup>54</sup> concerning hematoporphyrin may have no significance.

Cluver<sup>56</sup> combined the deficiency and the photodynamic theories when he found the diets of two groups of prisoners deficient in the pellagra-preventing factor. In those who were exposed to sunlight pellagra developed, while in those who were in the shade no symptoms appeared. Jobling and Arnold<sup>53</sup> produced evidence that the photodynamic theory and the parasitic theory could go hand in hand.

Sellards,<sup>57</sup> who studied photodynamic properties of sunlight in the tropics, believed that more investigations were necessary to correlate these properties with disease. He considered that photodynamic action is associated closely with fluorescence, but that they are not invariably associated.

At present it is evident that photodynamics in relation to pellagra is comparatively ill understood.

#### THE PARASITIC THEORY

The parasitic theory was given impetus by Sambon<sup>58</sup> after careful epidemiologic studies. Prior to that time organisms had been isolated from maize, but they were not satisfactorily correlated with pellagra. Sambon<sup>58</sup> noticed that pellagra did not attack indiscriminately all those who lived on maize; it had marked seasonal incidence; it was not transmitted by lactation, and conjugal pellagra had been reported. He suggested as a working hypothesis that pellagra was analogous to some of the protozoan diseases. Later Sambon found that men in the Venice arsenal did not have the malady, while the fishermen on other islands were pellagrous. He associated this observation with a biting fly and chose *Simulium* as a working hypothesis. Sambon's suggestions were not substantiated by the Illinois commission<sup>11</sup> and others. The important epidemiologic studies of Jobling and Peterson<sup>20</sup> correlated the disease with unscreened houses and with access of flies to human excreta and to human habitations. A decrease of pellagra was found with an improvement in the diet.

Numerous insects were investigated by Jennings and King,<sup>59</sup> but no positive results were obtained. The stable fly (*Stomoxys calcitrans*)

55. Meyer-Betz, F.: Deutsches Arch. f. klin. Med. **112**:476, 1913.

56. Cluver, E. H.: Brit. M. J. **2**:751, 1929.

57. Sellards, A.: J. M. Research **38**:293, 1918.

58. Sambon, L.: Brit. M. J. **2**:1272, 1905.

59. Jennings, A. H., and King, W. V.: Am. J. M. Sc. **146**:411, 1913.

was considered by them as the possible transmitter, but the Thompson-McFadden commission<sup>60</sup> was unsuccessful in finding the disease in monkeys bitten by these flies previously exposed to pellagrins. The streptobacillus of Tizzoni and de Angelis<sup>61</sup> found on maize and in the blood, organs and cerebrospinal fluid of pellagrous persons was not found by the Illinois commission,<sup>11</sup> Harris<sup>62</sup> and others. Salmon, Hays and Guerrant<sup>31</sup> isolated an organism from the cutaneous lesions, parenchymatous organs, intestinal submucosa and the cavities of the involved joints of their experimental animals. They fed the organism to rats and produced lesions from which the characteristic coccus was recovered. Susman<sup>63</sup> claimed to have found an anaerobe in the blood of a pellagrin. The fungus found in the stools of pellagrins by Jobling and Arnold<sup>53</sup> has been mentioned. All of this work should be repeated with more extensive observations, so that secondary invaders may be completely eliminated.

Feeding of pellagrous tissues and fluids to monkeys and inoculation of these animals with this material were unsuccessful in producing the disease, according to the Illinois commission<sup>11</sup> and Francis.<sup>64</sup> An important contribution was made by Harris<sup>65</sup> which has never been corroborated. Tissues from various parts of the bodies of pellagrins were mixed with equal amounts of normal salt solution. The coarsely filtered juice was passed through a Berkefeld filter and injected subcutaneously, intravenously and intracranially in large quantities into monkeys (*Macacus rhesus*). The three animals inoculated developed all of the principal clinical signs and symptoms and pathologic evidences found in pellagra. He concluded that the cause of the disease was a filtrable virus or a micro-organism that passed through a Berkefeld filter. Francis,<sup>64</sup> who experimented with ninety monkeys (*M. rhesus*), three baboons and one Java monkey, could not obtain similar results. Nevertheless, Harris produced the experimental pellagra that can be least questioned.

It has been noted that pellagra is associated with other diseases. The older writers found that tuberculosis was often present. Yang and Hu<sup>66</sup> and Turner<sup>67</sup> reported the occurrence of intestinal disease in

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60. Report of the Thompson-McFadden Pellagra Commission, New York, 1915.

61. Tizzoni, G., and de Angelis, G.: *Centralbl. f. Bakteriol.* **74**:219, 1914. Tizzoni, G.: *Nuove ricerche batteriologiche sulla pellagra*, *Rendic. r. Accad. d. sc. d. Ist. di Bologna* **12**:19, 1907-1908; cited by Marie (footnote 69).

62. Harris, H. F.: *Pellagra*, New York, The Macmillan Company, 1919.

63. Susman, W.: *Edinburgh M. J.* **34**:419, 1927.

64. Francis, E.: *Hygienic Laboratory Bulletin* 106, 1917, p. 81.

65. Harris, W. H.: *J. A. M. A.* **60**:1948, 1913.

66. Yang, C. S., and Hu, C. K.: *Nat. M. J., China* **16**:625, 1930.

67. Turner, R. H.: *Am. J. Trop. Med.* **9**:129, 1929.

certain pellagrins. Turner mentioned that a change in the intestinal flora might be an important factor in the etiology. The Illinois commission<sup>11</sup> found that the normal bacterial flora of the intestinal tract is disturbed in pellagrins, and that new forms appear, but no essential causal relationship of the organisms to the disease was demonstrated. The fungus found by Jobling and Arnold<sup>53</sup> to have photodynamic properties may have some significance.

The recent work of Matsumura, Kakinuma, Kawashima and others<sup>68</sup> in which they produced evidence that beriberi may be an infectious disease, gives further importance to the possibility that an organism is the causative factor in pellagra.

#### PATHOLOGIC ANATOMY

Since the etiology of pellagra has not been definitely determined, it is difficult to separate the exact pathologic changes due to pellagra. Emaciation has been reported often, but in the acute cases this is not always present. Fragility of the bones was observed by many of the early writers. Marie<sup>69</sup> reported fragility of the ribs in eighteen of forty-two cases. The work of Roberts<sup>70</sup> with use of the x-ray plate is to be commended, as he found rarefaction of the spongiosa and of the cortical layers in the bones of the hands, thus correlating the pathologic observations of the early pellagrologists. Atrophy of the muscles was found by Babès and Sion,<sup>71</sup> Marie<sup>69</sup> and Kozowsky.<sup>72</sup>

*Integument.*—The cutaneous lesions are present mainly on the surfaces exposed to sunlight, as those of the forearms, dorsa of the hands and feet and the back of the neck. Less commonly the lesions are present on the chest, face, perineum and scrotum. The symmetry of these lesions is important. Merk,<sup>73</sup> who described the gross changes, carefully noted the stages of erythema and of desquamation, but did not study the histology. The pathologic lesions were studied by Raymond,<sup>74</sup> Guard,<sup>75</sup> Ormsby and Singer,<sup>76</sup> Fiocco,<sup>77</sup> Kozowsky<sup>72</sup> and

68. Matsumura, S.; Kakinuma, G.; Kawashima, K., and others: J. A. M. A. **92**:1325, 1929.

69. Marie, A.: Lombroso's Trattato profilattico e clinico della pellagra (Turin, 1892), abridged edition, tr. from the French by Lavinder and Babcock, Columbia, S. C., 1910.

70. Roberts, S.: Pellagra, St. Louis, C. V. Mosby Company, 1914.

71. Babès, V., and Sion, V., in Nothnagel: Spezielle Pathologie und Therapie, Vienna, 1901, vol. 24, no. 2, pt. 3, p. 1.

72. Kozowsky: Arch. f. Psychiat. **49**:204, 556 and 873, 1912.

73. Merk, L.: Le manifestazioni cutanee della pellagra, Innsbruck, 1910.

74. Raymond, P.: Ann. de dermat. et syph. **10**:627, 1889.

75. Guard, F. B.: J. Exper. Med. **13**:98, 1911.

76. Ormsby, O. S., and Singer, H. D.: Report of the Pellagra Commission of the State of Illinois, Springfield, Illinois, State Printer, 1911, p. 16.

77. Fiocco: Atti d. quinto cong. pellagra ital. Bergamo, 1912, p. 305.



Babès and Sion<sup>71</sup> with varying results, principally because the stages of the disease are not given the importance deserved. Guard<sup>75</sup> correlated the changes in the skin with those normally produced by the action of sunlight, except that they are more marked. He differentiated the stages in his studies. Babès and Sion<sup>71</sup> found pigmentation, hyperkeratosis, hyperemia, edema and erythema, which evidently indicate stages. The pathologic changes in the skin were elucidated by MacNeal,<sup>78</sup> who stated that they are dependent on the stage of the disease. The early stage is represented by vascular injection and edema of the corium with a diffuse infiltration of wandering cells. Later edema and hyperplasia of the rete with regions of parakeratosis appear. Hyperkeratosis and hyperpigmentation are present as the malady advances. MacNeal<sup>78</sup> noted that the relationship of these processes varies according to the rapidity of the evolution and the severity of the eruption. Denton<sup>79</sup> summarized the cutaneous lesions as occurring in the following stages: injury, or fibrolytic stage; reaction, or dermatitis stage; repair; compensatory vascular ectasis, or erythematous stage, and the cicatricial, or atrophic, stage. This summary also makes clear the earlier reports. The fact that the various stages may be present in the same person at the same time has not been emphasized as much as it should be.

*The Nervous System.*—As varying degrees of pathologic changes had been found in the nervous system, MacNeal<sup>78</sup> believed that these lesions should be studied in relation to the stage of the disease. The pathologic reports by Babès and Sion,<sup>71</sup> Marie,<sup>69</sup> the Illinois commission,<sup>11</sup> Kozowsky,<sup>72</sup> Sandwith,<sup>80</sup> Sundwall,<sup>81</sup> Castellani and Chalmers,<sup>82</sup> Harris,<sup>62</sup> Winkelman<sup>83</sup> and Pentschew<sup>84</sup> did not adequately emphasize the stage and its relationship to the pathologic changes. Marie<sup>69</sup> noted atrophy of the brain in cases of long standing mental derangement. Singer and Pollock<sup>85</sup> did not find that the gross morbid anatomy was characteristic, although they found edema and thickening of the meninges. However, they differentiated between acute changes, consisting in chromatolysis of nerve cells, satellitosis, astrocytosis and the presence of ameboid glial cells, and chronic changes, such as fatty and fibrinoid degenerations, regressive changes of the nerve cells, chronic Nissl

78. MacNeal, W. J.: *Am. J. M. Sc.* **161**:469, 1921.

79. Denton, J.: *Am. J. Trop. Med.* **5**:173, 1925.

80. Sandwith, F. M.: *J. Path. & Bact.* **7**:460, 1901.

81. Sundwall, J.: *Hygienic Laboratory Bulletin* 106, 1917, p. 50.

82. Castellani, A., and Chalmers, A. J.: *Manual of Tropical Medicine*, New York, William Wood & Company, 1919.

83. Winkelman, N. W.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **102**:38, 1926.

84. Pentschew, A.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **118**:17, 1929.

85. Singer, H. D., and Pollock, L. J.: *Arch. Int. Med.* **11**:565, 1913.

changes of the nerve cells, increase of glial fibers, permanent destruction of nerve fibers and a marked increase of amyloid bodies. Central neuritis was present during or soon after an acute attack. Mott<sup>86</sup> found degenerated fibers of nerves; these were found generally diffused and scattered throughout the white matter of the cord, but were most marked in the posterolateral and the posteriomedian columns. The cells of all of the posterior spinal ganglions showed, in varying degrees, marked chromatolysis, swelling, and disappearance of Nissl granules except at the periphery. Wherever the nervous system was examined, the cells that normally had a Nissl pattern seemed to show a change in the nature of a disappearance of the granules. Fibril changes occurred in the cells of the anterior horns and the cells of Purkinje, and also in Betz' cells, but to a less degree. Castellani and Chalmers<sup>82</sup> wrote that degeneration occurred in the cells of the spinal ganglions, in those of the posterior cornu and in those of Clarke's column, with Nissl bodies and fibrils disappearing in the posterior roots, in Lissauer's tract, in Burdock's column, in Goll's column and less commonly in the lateral columns. Harris,<sup>62</sup> who reviewed the literature extensively, stated that in the medullar and cerebral cortex areas of sclerotic changes were present, resembling those in the cord. The pyramidal cells suffered most. The changes in the spinal and sympathetic ganglions were similar to those found in the central nervous system, although usually not so marked. The early arteriosclerotic changes in the brain reported by Denton<sup>79</sup> were of importance, as he found them in patients under 25 years of age. He concluded that the changes in the central nervous system were not in direct proportion to the acuteness and severity of the disease, but that they were more pronounced in chronic pellagrins. His statements probably represent the truth, for many of his observations were similar to those of other pathologists, but his material was somewhat limited.

*The Gastro-Intestinal Tract.*—Harris<sup>62</sup> reported that the red and inflamed tongue often became ulcerated. Hypertrophy of the superficial lingual tissues occurred if the attacks were repeated. This hypertrophy was followed by shrinkage. Similar changes were present in the gums, lips and buccal mucous membrane. Denton<sup>79</sup> found that in the pharynx, tongue and esophagus the repair of the membrana propria was imperfect, and that the epithelium regenerated in a thin layer. He found that this reaction was essentially correlated with that in the skin. MacNeal emphasized that hyperemia of the intestines might alternate with a normal condition of the bowel. I later observed this change. MacNeal<sup>78</sup> noted that in the earlier lesions there were vascular

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86. Mott, F. W.: Brit. M. J. 2:4, 1913.

engorgement, hyperplasia of the lymphoid tissue, and edema and infiltration of the mucosa with wandering cells. In the later lesions collections of wandering cells extended from the epithelial surface into the submucosa and along the sheaths of blood vessels into the muscular coats. The inflammation of the large intestine reported by Lynch<sup>87</sup> was practically limited to the colon. Microscopically, the mucosa was hyperemic; the surface epithelium was degenerated and desquamated; there was an increased number of interglandular mononuclears and connective tissue cells, and there was marked degeneration of the epithelium of the glands with cystic dilation. The mouth and the neck of the gland underwent hyaline changes with occlusion by degenerated and cast-off epithelium, which he believed to be the beginning of the process leading to active inflammation of the mucosa, submucosa and other coats to thrombosis of mucosal and submucosal veins and lymphatics and to necrosis with ulceration. In chronic cases Lynch<sup>87</sup> found sclerosis of the small blood vessels out of proportion to the arteriosclerosis elsewhere. Denton<sup>79</sup> described changes such as cellular infiltrations, varying grades of acute inflammation, dilated blood vessels and changes in the sizes and shapes of surface epithelial cells, yet he did not consider them absolutely specific. Lynch<sup>87</sup> believed that the changes that he described in the colon were characteristic of pellagra.

*Liver.*—Babès and Sion,<sup>71</sup> de Giovanni,<sup>88</sup> Sundwall,<sup>81</sup> Crutchfield,<sup>89</sup> Kozowsky,<sup>72</sup> Denton<sup>79</sup> and the Illinois commission<sup>11</sup> reported fatty changes in the liver. A small liver was noted by Marie<sup>69</sup> and Harris.<sup>62</sup> DeGiovanni<sup>88</sup> stated that it remained permanently smaller after an attack. The Illinois commission<sup>11</sup> found inflammation of a low grade in the portal connective tissue in the interlobular septums, engorgement of the intralobular capillaries and fatty degeneration around the lobule in every case. This commission concluded that some of the specimens suggested an early stage of acute yellow atrophy or the more acute forms of alcoholic cirrhosis. Focal necrosis was noted by Denton.<sup>79</sup> From these reports there is indication of a toxic effect, although adequate proof of this is lacking.

*Pancreas.*—The reports of changes in the pancreas are few, and the changes are not characteristic. Raubitschek,<sup>90</sup> who carefully reviewed the literature, found no characteristic changes.

*Circulatory System.*—Atrophy of the heart was found by Marie<sup>69</sup> and by Babès and Sion.<sup>71</sup> Babès and Sion<sup>71</sup> found atheromatous

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87. Lynch, K. M.: *Internat. Clin.* **3**:130, 1930.

88. de Giovanni: *Patologia del simpatica*, Milano, 1896; cited by Harris (foot-note 62).

89. Crutchfield, E. D.: *Arch. Dermat. & Syph.* **17**:650, 1928.

90. Raubitschek, H.: *Ergebn. d. allg. Path. u. path. Anat.* **18**:1, 1915.

plaques in the aorta, and the Illinois commission<sup>11</sup> reported hyaline changes in the intima of the blood vessels. Kozowsky,<sup>72</sup> in his reports of arteriosclerosis, eliminated all cases in persons past middle life. When he found hyalinization and thickening of the walls of the blood vessels, changes that were especially noticeable in the walls of the smaller vessels, his careful observations could be correlated with those in old age. The work of Kozowsky<sup>72</sup> is important. The arteriosclerosis reported by Harris<sup>62</sup> might have some significance.

*Respiratory System.*—The pathologic changes reported, such as pneumonia, emphysema, edema, pleurisy, etc., do not appear to have any relationship to pellagra. Marie<sup>69</sup> noted the pulmonary tuberculosis of which frequent mention had been made by the older writers.

*Lymphatic System.*—Marie,<sup>69</sup> Tuczek,<sup>91</sup> Babès and Sion<sup>71</sup> and Sundwall<sup>81</sup> found atrophy of the spleen. Kozowsky<sup>72</sup> reported the characteristic sclerotic vessels, which were more pronounced in the spleen than in the lymph nodes. Babès and Sion<sup>71</sup> noted tabes mesenterica associated with pellagra, especially in children.

*Genito-Urinary System.*—Atrophy of the kidneys was noted by Babès and Sion<sup>71</sup> and Marie.<sup>69</sup> Kozowsky<sup>72</sup> related destruction of tubules and thickening of Bowman's capsule and of interstitial tissues to arteriosclerosis. No noteworthy gross or microscopic pathologic changes in the genitalia could be related to pellagra.

*Endocrine Glands.*—The reports of changes in the suprarenal glands, hypophysis and thyroid gland are few, and the changes could scarcely be correlated with pellagra. Susman<sup>92</sup> found that in the suprarenals in five cases the cortical lipid was scant or absent, but that a secondary infection would have to be eliminated. Morse<sup>93</sup> reported lesions resembling chronic productive thyroiditis with the follicles undergoing a compensatory reaction. The fibrosis reported to have occurred in the thyroid gland should encourage further observations, as this gland has not been given the study by pathologists that it should have.

*Eyes.*—The atrophy of the choroidal pigment found by Rampoldi<sup>94</sup> and the early cataracts reported by Welton<sup>95</sup> might be related to the early changes in senility. Elimination of older patients would have added value to the observations of Welton.<sup>95</sup>

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91. Tuczek: A Dictionary of Psychological Medicine, Philadelphia, D. H. Tuke, 1892; cited by Marie (footnote 69).

92. Susman, W.: Tr. Roy. Soc. Trop. Med. & Hyg. **24**:23, 1930.

93. Morse, P. F.: J. Lab. & Clin. Med. **1**:217, 1916.

94. Rampoldi, R.: Ann. di ottal. e clin. ocul. **14**:99, 1885.

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## SUMMARY

A scrutiny of the literature published during the last thirty years reveals the prominence of the theory that a deficient diet is the cause of pellagra (which is an outgrowth of the conception that the disease comes from the consumption of spoiled maize). The theory that pellagra is caused by a deficient diet has advanced recently to the theory that a deficiency of "vitamin G" is the cause. At present evidence accumulates that vitamin G is composed of two factors. Whether iron or cane sugar is related to the etiology is a question for the future. The significance of photodynamic action in pellagra still has its place; because of the seasonal variations noted in lesions of the experimental animals suffering from lack of the antipellagric vitamin; also because a fungus having fluorescent properties has been isolated from the stools of pellagrins.

Pathologic changes similar to those in senility are found in pellagrins. Erythema, edema, hyperkeratosis and hyperpigmentation of the skin, degenerative changes in the nervous system, and hyperemia, inflammation and repair of the upper gastro-intestinal tract and of the colon are the most important lesions in pellagra.

Since the exact etiologic factor in pellagra is still questioned, difficulties arise in the interpretation of the pathologic changes due to the disease.

# THE ETIOLOGY OF CANCER

## I. TISSUE TRANSPLANTATION AND PARASITISM \*

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OMAHA

In the following general and more or less critical review of the subject of the etiology of cancer, no claim is made for completeness. Even if it were desirable to include references to all that has been contributed to the subject, the publications are too numerous and too scattered to permit the hope of such an absolutely complete survey. I hope, however, and trust, that few articles of major importance have been omitted here.

Owing to the unavoidable length of a summary even as condensed as this, it has become necessary to divide it into four parts. The first deals with the results of tissue transplantation in their relations to cancer, and with the problem of parasitism in cancer. The second will deal with the general subject of irritation and cancer. A third will take up various miscellaneous features of the etiology of cancer—its relations to neurotrophic changes, to nuclear aberration and to heredity, and the factors of general or local character affecting the growth and survival of already initiated tumors. The fourth and final part will deal with metabolism in its relations to the etiology of cancer.

It is not proposed to discuss here, except incidentally to facts as revealed by experimental study, the more or less classic histogenic theories of the origin of cancer; while they represent brilliant feats of deductive reasoning, they are seldom of such character as to admit of experimental control, and the expanding knowledge of the phenomena of cancer development derived largely from experimental study bids fair to supplant them entirely.

### TISSUE TRANSPLANTATION

*Heterologous Transfer of Tumor Tissue.*—To the extent that the earlier stages of the etiologic study of cancer were a matter of experimental investigation, as aside from speculations based largely on morphologic observation, this work was devoted almost wholly to the search for a possible parasitic cause. Even the earlier experiments on the

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transferability of cancer had in the background the idea of causation by transfer of a parasite rather than of the cancer itself. As early as 1840 Langenbeck reported that 60 days after the injection of human cancerous fluid into a dog the animal was found to have tumor nodules in the lung. In 1843 Klencke averred that he had confirmed Langenbeck's work, without, however, publishing any details. Somewhat similar results were reported by Lebert in 1851, but in 1867 he and Wyss were unsuccessful in transferring human tumors to rabbits or to guinea-pigs; in the same article they described the appearance of local tumors in the neck of a dog after the injection there of fluid from an ulcerated human esophageal cancer—lesions which, from their description, were almost certainly of acute inflammatory character. In that same year Goujon alleged that he had succeeded in transferring a human testicular cancer to the white rat, in which the observed tumors had no relation to the site of inoculation, and to the guinea-pig. In 1874 Quinquand found in a fowl into which he had injected a small quantity of a cancer of human liver multiple pulmonary masses, which he regarded as cancerous. Thiroloix in 1892 observed palpable masses in the abdomen of a rabbit which had been inoculated intraperitoneally with mammary cancer from a white mouse; Francotte and de Rechter the same year believed that they had produced cancer in mice by the repeated injection of human cancerous material, and in the following year Mayet observed in a rat the appearance of multiple visceral cancers a year after the injection of fluid from glycerinated and macerated human cancers into a number of those animals. He later (1901 and 1905) added to this similar reports of additional results, with alleged success in 5 of 52 attempts, and 4 apparent successes with human material introduced into dogs. In 1894 Boinet reported that he had been successful twice in transferring human cancer to the rat, and once to the rabbit, in a total of 60 attempts. Jürgen in 1896 observed generalized melanomatosis transmissible by homotransplantation in rabbits inoculated with human melanotic tumor, and in 1898 Queyrat found that pigmented intraperitoneal masses were formed in macacus monkeys after similar inoculation. Dagonet in 1903 believed that he had successfully inoculated an epithelioma of the human penis into the peritoneal cavity of the white rat, in which, 17 months later, he found disseminated intra-abdominal lesions, which he stated to be of the same type as the injected tumor. Together with Mauclaire he reported in 1904 the successful inoculation of human rectal adenocarcinoma into the rat, with the development of tumors, though of dissimilar type, in that animal which were transplantable for another generation.

Of these earlier alleged successful transfers of cancer from one species to another, none can be regarded as of undoubted authenticity. Some of the observed lesions were possibly cancerous, but, as was

believed by Virchow of Langenbeck's tumor, of spontaneous rather than induced origin. And some with even greater certainty were masses of inflammatory character, as may occasionally be inferred from the descriptions given by the experimenters themselves.

In 1905 there appeared a report by Lewin of what is by far the best authenticated and most carefully considered of the various attempts at heterotransfer of a tumor. Two months after the introduction into the peritoneal cavity of a dog of material from an unusually malignant human ovarian cancer the animal showed local masses of a morphologic type that was not definitely neoplastic, but that differed from the usual granulomas in that the tissue was inoculable into other dogs. Lewin was unable to classify the lesion exactly, but in 1907 he reported the development of inoculable granulation tissue about a bit of human cervical cancer embedded in the white rat.

Of more recent work, there is the report by Robertson, in 1909, of the appearance of malignant tumors of miscellaneous types in 7 of 30 mice fed with exudative material from human cancer. In 1911 Gargano reported a number of cases of alleged production of tumor in mice after inoculation with human material, the lesions, according to him, being originally carcinomatous, later changing to sarcoma—the inference being that they were granulomas. Strauch in 1913 believed he was successful in transferring a very malignant mouse carcinoma to 50 per cent of his rabbits, as well as in the transfer of a human cancer to a macacus monkey. But a comprehensive repetition of his work by Apolant and Bierbaum led them to the conclusion that the lesions observed by Strauch were inflammatory. In 1923 Nather reported the transfer of cancer from mouse to rabbit by the repeated injection of emulsions of tumors, and Heidenhain in 1928 stated that in mice into which autolysates of the malignant tumors of man, cattle and mice had been injected there was an unduly high frequency of tumor development. The tumors were of diverse character; as a rule there was no relationship of the site of the tumor to that of the injection, and the rate of incidence was not sufficiently high to exclude the possibility of spontaneous occurrence.

Actually there is a much greater volume of evidence to show that heterotransfer of tumors does not take place, except in certain circumstances that infringe rather slightly on the bounds of tissue specificity. Among those reporting unsuccessful attempts at the transfer of human cancer to various laboratory animals are Fischer in 1882, Senn and Senger in 1888, Klebs in 1890, Morau in 1891, Fischel, Duplay and Cazin in 1892, Kopfstein in 1893, Curtis in 1899, McConnell in 1908, Vidal in 1909, Bartkiewicz and Leotta in 1910, Williams in 1911, Fischel in 1922, Kurtzahn in 1926 and Tinozzi in 1929. The foregoing list of investigators who reported failures in the attempted heterotransplantation of



tumors is by no means exhaustive—numerous earlier efforts, not cited here, are given in reviews by Pilliet and by Vischer. Suffice it to say that the altogether predominant evidence favors the view that the successful transfer of malignant tumors, with certain special exceptions, may be accomplished only in circumstances that permit the continued survival and growth of the tumor cell itself, and of course in these circumstances the transfer of the tumor to a new host is actually that, and not the transfer of a causative agent of possibly parasitic character. Careful scrutiny of the cases recorded in the foregoing paragraphs which do not accord with this view will show, practically without exception, that the allegedly positive results cannot be accepted without some skepticism. In many the tumor-like lesions were almost certainly of inflammatory character; in others the growths would appear to have been spontaneous tumors, and the very plenitude of positive results reported by some experimenters are so unlike the results of most other investigators as to make them of doubtful authenticity.

Among the special exceptions mentioned must be included a group of dog tumors that show certain peculiarities in the way of transmissibility. There have been reported a considerable number of transferable tumors of this animal, including some of the earliest successful efforts at transplantation of tumors. Smith and Washbourn reported in 1897 the occurrence in dogs of tumors, apparently round cell sarcomas, which were readily propagated by implantation and which were transmissible by coitus. In 1902 White, with another instance of this type, reported that the tumors were lymphosarcomas, and again observed transmission by coitus. In 1904 Sticker described a similar tumor with a high degree of transmissibility by inoculation, and in 1906 Beebe and Ewing reported on another that combined the features of high inoculability and venereal transmission. In that year Sticker found that his tumor was inoculable into foxes, although not into a miscellaneous series of utterly unrelated animals. That this was not a matter of cellular transfer was suggested by Wade in 1907, who regarded the disease as a general infection with localized lesions of sarcomatous type; while the virus was almost certainly intracellular, it was not filtrable, a finding that was confirmed by Beebe and Ewing. In 1909 Sticker observed in the breast of a bitch that had been inoculated with one of these sarcomas (in this case of spindle cell type) the development of a slowly growing, but metastasizing, adenocarcinoma; and in 1912 von Dungern was able to show definitely that in the transfer of these sarcomas to foxes cellular implantation was not concerned, by the failure of the induced fox tumors to produce dog antibodies after injection into other foxes—unlike the result that followed the injection of the original dog tumor tissue.

*Homologous Transfer of Tumor Tissue.*—Although, in general, experimental heterotransplantation has yielded negative results, the situation is of course quite different as concerns the transfer of cancer within the host species. Even before the successful artificial transplantation of tumors, clinical observation had shown that such transplantation took place within the individual, even if the phenomena of metastasis were not sufficient to establish this. Natural transplantation by implantation was reported by Virchow in 1863, Kaufmann in 1879, Nicasse in 1883, Kraske and Lucke in 1884, Geissler in 1893, Claude and Pilliet in 1895, Cabot in 1901 and Wilmanns in 1904, and by intentional surgical procedures by Hahn in 1888 and Cornil in 1892. From these, it could be surmised logically that transfer from one animal to another sufficiently akin should be possible, and although the limited number of attempts at this with human beings—such attempts were reported by Senn in 1888 and by Kurtzahn in 1926, each using himself as the subject—were unsuccessful, as early as 1876 Novinsky reported the transfer of a cancer of the nose from one dog to another, with doubtful success in the second generation. A similar attempt reported by Mathis in 1885 was not unquestionably successful, but in 1889 both Wehr and Hanau were able to secure successful transfers, the one with a squamous cell carcinoma of the dog, the other with a similar tumor of the rat, which he transplanted for 3 generations. In the next year von Eiselberg reported the transfer of a fibrosarcoma from one rat to another, and in 1891 Morau announced the transplantation of a mouse carcinoma, which by 1893 had apparently attained 17 generations. Firket in 1892 succeeded with a rat sarcoma, and Velich in 1898 with a similar tumor, in this case for 9 generations. Although these earlier successes did not attract widespread attention, the almost simultaneous work of Loeb, with a sarcoma of the thyroid gland of the white rat, which he was able to propagate indefinitely, and of Jensen, with a carcinomatous tumor of the mouse, which by 1903 he had transplanted for 19 generations, established beyond question the feasibility of homotransplantation of malignant tumors. But from the point of view of the etiology of tumors, neither these nor the numerous subsequent successful transfers have been of direct value, since the phenomena of transplantation are essentially those of artificial metastasis of already established tumors. The extent to which they have been of value in this connection has been to supply a constant and accessible source of tumor material and to afford some knowledge of the conditions permitting tumor growth.

Strictly speaking, tumor transplantation is not entirely limited to animals of the same species. Occasional cases are not subject to this restriction; von Dungern and Coca found a sarcoma of the hare which could be transplanted into rabbits without loss of its original hare

specificity, and Fujinami and Hatano, a fowl sarcoma that would survive in the duck. Flexner reported the temporary growth of human teratomatous tissue inoculated into monkeys, and Putnoky very recently published an account of the transfer of the Ehrlich mouse carcinoma to the rat, with successful retransfer back to mice. The successful growth of tumor tissue in foreign embryos, as reported by Murphy, Kiyono, Kawakami and Sueyasu, Stevenson, Bullock and Gheorgiu, does not materially affect the problem, as Murphy showed that such growth ceased in the later periods of embryonic life, when tissue specificity appeared. This same lack of specificity evidently applies to the heterologous implantation of tumors into brain tissue, as reported by Murphy, and into the eyeball, as observed by Hegner. Instances of heterologous transfer after modification of the new host, as reported by Roskin for fowl sarcoma introduced into the mouse after reticulo-endothelial blockage, are presumably to be interpreted, too, as instances of survival of the introduced tumor cells and their descendants. Other, less definite means of modification of the new host have been attempted, in some cases with alleged success. Funk in 1915 was able to implant an inoculable chondroma of the mouse into the rat after feeding the latter with the tumor tissue prior to inoculation, and Nagayo and Wago in 1921 also reported the successful heterotransplantation, though to a limited degree, of tumor after preliminary preparation of the new host. Keysser in 1920 reported the appearance of a sarcoma in 1 of a number of mice inoculated with the emulsion of a rapidly growing human testicular tumor, after they had been given injections of autolysates of human sarcomas. And Flaszen and Wachtel reported that human cancer could be successfully implanted in white mice after preliminary alkalinization; but in this case the photomicrographs are particularly unconvincing.

*Transplantation of Embryonic Tissues.*—It may be well to include at this point the attempts at causation of tumors by the introduction of embryonic tissues, with their obvious relationship to Cohnheim's hypothesis. Such implantation has been attempted by numerous observers, Leopold in 1881, Lwoff in 1883, Zahn in 1884, Fere in 1897, Traina in 1902, Fraenkel in 1903, Wilms in 1904, Petrow, Rössle and von Hippel in 1906, Bogoljuboff in 1908, Askanazy in 1907 and 1909, Neuhäuser in 1909 and 1911, Shattock, Seligmann and Dudgeon in 1909-1910, von Tiesenhausen in 1909, Freund and also Rous in 1911, Gargano in 1914, Ikematsu in 1923, Wereschinski in 1924, Skubisrewski in 1925 and 1928, Ide and Loewenthal in 1928. The result of all of these attempts, with occasional exceptions, was the occurrence of growth for a limited period, followed either by persistence without progression or by eventual regression. Among the exceptions are those of: Petrow, who found that the growths obtained in his

animals showed some slight inoculability into other animals of the same species; Askanazy, who observed the development in an experimental teratoid of a sarcoma inoculable for 1 generation only, and according to his later report, of teratoids which could themselves be transplanted for 2 or 3 generations; Neuhäuser, who after the introduction of fetal adrenal tissue into the kidney of an adult rabbit, observed the appearance of a tumor first regarded as a hypernephroma, later as adenomatous; von Tiesenhausen and Skubisrewski, who both saw in some of their teratoids sarcoma-like inclusions, which were, however, without other evidence of progressive growth; and Wereschinsky, who believed that he had obtained a spindle cell sarcoma in 1 case after the implantation of fetal guinea-pig tissue in the adult animal. Belogolowy, too, observed what he regarded as a sarcomatous growth of implanted amphibian embryonic tissue, a belief in which he was supported by Roux. But repetition of his experiments by Piette, Anders, Bierich and Teutschlaender led all these to the conclusion that to the extent that proliferation occurred it was of granulomatous character. The mass of evidence would seem to be sufficient to establish the fact that embryonic displacement alone is not sufficient to cause malignant growth, at least with displacement such as may be achieved experimentally. The exceptional instances in which cancer has followed such experimental displacement have thrown no light on the problem of the causation of the tumor, except in some more complicated experiments, to be discussed later, in which to the displacement was added accessory action of one sort or another.

*Transplantation of Adult Tissues.*—Similarly, a number of experiments involving the transplantation of adult tissues have been undertaken in an effort to check Ribbert's hypothesis of postembryonic displacement as a factor in the genesis of tumor. Even before the publication of Ribbert's hypothesis, Schweningen in 1886 had reported that transplanted epithelium showed only restricted growth; Levin in 1901 likewise observed some growth of transplanted skin, but not of other tissues; Marnoch in that year, Nichols in 1904-1905 and von Lamezan in 1913 observed at most only limited proliferation after transplantation. Only one or two reported experiments stand in contradiction to these findings. Lack in 1900 inserted ovarian scrapings of rabbits into their peritoneal cavities, and in 1 of 2 surviving animals found a generalized implantation of epithelium throughout the peritoneum, with growths in the liver, pleura and lungs. And in 1912 Stieve found that in 1 of 46 guinea-pigs in which the effort was made to transplant the granulation tissue produced in response to the injection of kieselguhr, the tissue on implantation was capable of sustained, independent and infiltrative growth.

## PARASITISM

*Protozoa.*—While the earlier attempts at the transfer of cancer were based on the idea of demonstrating its parasitic cause, numerous more direct efforts at the discovery of a conjectured parasite were being made. Among these were the numerous attempts to discover protozoan organisms, and the earlier literature on the etiology of cancer abounds with the claims of those who believed that such parasites had been discovered. For the most part, these claims were based wholly on morphologic observation. Unusual inclusion bodies in cancer had been observed by Müller as far back as 1839, by Virchow and by Bruch in 1847, and by Hannover in 1852. Virchow, at least, interpreted these as degenerative inclusions, and it was not until 1888 that Pfeiffer announced the belief that among them were forms that could be regarded as protozoa. In the following year, Darier, Albarran, Malassez and Thoma reported what they regarded as sporozoa or coccidia in human cancers, with as a rule a claim for their etiologic relationship to the disease. Darier based his claim largely on a study of Paget's disease of the breast, and in 1890 Hutchinson and Wickham believed that they were able to substantiate his claim. In this year other reports of supposed protozoa in cancer were made by Hache, van Heukelom, Sibley, Sjöbring, Vincent and again by Pfeiffer. Not all of these reports were accompanied by claims of causal association—this is true throughout this entire field of cancer research—but enough positive statements were made to excite a considerable volume of adverse comment. Cazin, Eberth, Klebs and Schütz were among the first to deny the parasitic nature of these inclusions, and Borrel particularly insisted on their degenerative character. Possibly as a result of these views two reports of protozoon-like inclusions made in 1891 by Bowlby and Stroebe were reserved in their interpretations, as was the case with a similar report made by Kürsteiner in 1893, and their identification of the bodies as parasites was at most only tentative. In the meantime there was an increasing mass of adverse opinion, Fabre-Domergue, Boeck, Firket, Kanthack, Piffard, Pilliet, Ribbert and Steinhaus all taking this view. Since Delepine had suggested that the mildly stimulant action of many protozoa would well serve to explain the phenomena of cancer, Shattock and Ballance attempted cultivation experiments with cancerous tissues, along with the inoculation of cancers into lower animals, as well as of coccidia into rabbits, with entirely negative results. During 1892 the discussion continued unabated, Boyce, Cazin, Fabre-Domergue, Flexner, Karg, Müller, Kosinsky, Raum and Virchow maintaining that the disputed inclusions had their origin in phagocytic or degenerative processes, and Delepine becoming more doubtful of their micro-organic character. Steinhaus, on the other hand, was disposed to admit the parasitic nature

of some of the inclusions, and Foa, Malassez, Metchnikoff, Soudakewitch, Sawtschenko, Ruffer, Walker and Podwyssozki either reported the identification of protozoan parasites or claimed this character for organisms previously described. During the next few years the negative view was argued by Boyce and Giles, Cazin and Duplay, Claessen, Gibbes, Hebb, Ohlmacher, Pianese, Petersen, Rosenthal, Snow, Fabre-Domergue, Cornil, Steinhaus, Ribbert, Adler and Borrel, and with certain reservations by Power, Massari and Ferroni. But at the same time renewed claims were being made by Burchardt, Cattle, Clarke, Galloway, Korotneff, Pawlowsky, Ruffer, Ssudakewitsch, Foa, Kahane, Kurloff, Nepveu, Steven and Brown, Vedeler, Smith, Power, Busquet, Bosc, Gaylord, Eisen, Sjöbring, Schüller, Jürgen, von Leyden, Jaboulay and Feinberg. With almost all of these alleged parasites, the only evidence of their existence was morphologic observation, and occasionally this was the subject of severe criticism—the organisms of Schüller, for instance, being identified, perhaps with justice, as particles of cork. However, Gaylord believed that the inoculation of his protozoa into guinea-pigs was occasionally followed by the appearance of cancerous lesions, and Sjöbring that similar results followed when his parasites were injected into white mice. Even later claims have been made for protozoan parasitism in cancer by Saul, Robertson, Jaboulay, Awerinzew, Elmassian, Clarke, Koch and, as lately as 1928, by Krug. Later exponents of the adverse view have been Nösske, Honda, Calkins, Borrel, Apolant and Embden, Klimenko, Bastian, Rosenberger, Unna, Greenough, Farmer, Moore and Walker, Blum, Grünbaum and Podwyssozko, who in 1909 acknowledged the degenerative character of organisms previously regarded by him as parasites, Brown, McDonagh, Lewis, McConnell and Lang, Deetjen and Machiarulo. While there was no particular harmony in the ideas of just what the inclusions might be, all the aforementioned investigators were insistent that the identification of the inclusion bodies as parasites was incorrect or at least conjectural, and their etiologic significance even more so.

More carefully considered than the attempts at identification of a parasite almost solely on morphologic grounds was the work of O. Schmidt, who in 1903 reported the observation in cancer of a sporulating parasite, with ciliated and ameboid stages, which in his experience could be grown on strains of *Mucor racemosus*. In later publications he announced that inoculation of infected colonies of *Mucor* into laboratory animals was followed by the appearance of malignant tumors—sarcomas in rats and mice and 1 carcinoma in the mouse. In addition, he found that injection of killed cultures had an immunizing effect against implanted sarcomas in rats. But Schuberg in 1906 disagreed as to the protozoan character of the alleged organism; Baisch in 1908

observed only one tumor in 70 survivors of 100 mice inoculated with Schmidt's organism, and quite failed to get any evidence of immunizing action; von Wasilewski and Wülker in 1912 could not find the protozoon in strains of *Mucor* obtained from Schmidt, nor in attempts of their own at its isolation; nor could they produce tumors in animals by experiments reproducing those of Schmidt as closely as possible. On the other hand, Rehorn in 1928 cultivated strains of *Mucor* from 2 human tumors, and in 70 rats inoculated with these observed the appearance of 2 cases of tumor.

Alleged successful causation of malignant tumors in animals by the injection of supposedly protozoan organisms was also reported by Walker in 1911, and by Flynn in 1922. The former worked with an organism obtained from the earthworm, which he regarded as the intermediate host; his photomicrographs show what appear to be granulomas. As to Flynn's work, the report is not sufficiently detailed to permit discussion. Von Calcar reported in some detail experiments to prove the protozoan origin of cancer: observations of proliferative growth in the livers of dogs, occurring only about such individual trematodes as harbor protozoa (*Metorrrchis truncatus*); lessened incidence of tar cancer in mice shielded from possible protozoan infection; induction of cancers in achylic dogs by allegedly infected material, etc.

The discovery that myxameboid organisms, parasitic in plants, were capable of exciting these to redundant tumor-like growth, served as a stimulus to the identification of similar organisms as the cause of tumors in animals. The protozon reported by Pfeiffer in 1890 was regarded by him as analogous in appearance to *Synchytrium taraxaci*, and that of von Leyden was compared with *Plasmodiophora brassicae*; Robertson and Wade and Fink in 1905 fancied a like similarity between apparent observed organisms and that parasite, as had Gaylord in 1902. Behla, who adduced the parasitic nature of cancer on indirect grounds, was inclined to implicate organisms of this type. However, although Podwyssotzkoi in 1900 got what he regarded as giant cell sarcomas in rabbits and guinea-pigs after their inoculation with infected leaves, in 1905 Löwenthal reported negative results from inoculation experiments, and Saul in 1906 obtained at most epithelial and connective tissue hyperplasia suggestive of chronic inflammation. Von Prowazek, after close study, was unable to find any similarity between the suspected cancer inclusions and *P. brassicae*.

The assertion made by Adamkiewicz in 1893 to the effect that the cancer cell itself is a foreign protozoan parasite, which was persistently advocated by him as lately as 1911, evoked some of the harshest criticism (Kinscherf and Bartsch, Paltauf and von Hansemann) that appears in the annals of cancer investigation. A similar view was advanced by Butlin, but received no wider acceptance. The idea that the

cancer cell itself is a parasite, not necessarily of foreign origin, has been advanced with some frequency—by Bard, Debove, Raymond, Tyzzer, Walsh and Critzmann, the latter with the additional conception that its origin results from the inclusion of one impregnated ovum within another. The conception of the cancer cell as a parasite, without any suggestion of how that parasitism is achieved, simply serves as a brief and more or less accurate characterization of its behavior, and is of course without bearing on etiology.

This statement does not apply to the theory of Kelling, who regarded cancer as the result of implantation in the organism of foreign embryonic cells, the character of which he believed he could identify by suitable immune reactions; he reported the experimental induction of tumors, though in a small proportion of his animals, by methods which he regarded as confirming his views. In an analysis of Kelling's work by von Dungern, it was pointed out that the immune reactions which he reported were much more plausibly explained as being of nonspecific character. Lubarsch, although he could not accept Kelling's theory, believed that in some of the latter's experiments there was evidence of transfer of tumor-inducing substances, but Ribbert refused completely to credit Kelling's reports of apparently successful induction of tumors.

*Bacteria and Other Vegetable Parasites.*—Almost coincident with the reported occurrence of protozoan parasites in cancerous growths were a number of efforts to implicate various bacteria as their cause. In 1887 Scheurlen described the isolation from 10 cases of human cancer of a spore-forming bacillus with which he believed he could cause carcinoma of the breast in dogs. Rappin, in the same year, similarly obtained a diplococcus, which in 1 instance caused what he described as a cancer of the breast in an inoculated rabbit; Schill reported the occurrence in both carcinomas and sarcomas of bipunctate organisms, for which, however, he made no etiologic claim. Meanwhile, Ballance and Shattock, although professed believers in the infectious origin of tumors, reported that in the great majority of cancers studied by them the bacteriologic observations were consistently negative. In 1888 Freire believed that he could confirm Scheurlen's findings, and even claimed to have anticipated them; Francke, too, agreed with Scheurlen as to the presence of the bacillus in human tumors. But Senger, Pfeiffer and Baumgarten, all working with this bacillus, were unanimous in regarding it as a mere contaminant, basing their conclusions on its cultural characteristics, on the fact that it appeared not infrequently as a contaminating organism on their culture mediums, and on its only occasional presence in tumors which were almost without exception ulcerated. Makara reported that systemic bacteriologic study of cancers only rarely showed the presence of bacteria, even as



accidental invaders, while Bernabei, who recovered from tumors organisms similar to those described by Schill, found that their only effect on inoculation was the production of a rapidly fatal marasmus and cachexia. Rosenthal, and a little later Sanquirico, after studies of Scheurlen's bacillus, denied that it could have any causal relationship. That organisms could easily occur in cancer without having necessarily any bearing on its cause was indicated by Gley and Charrin, who found a rabbit tumor heavily infected with *Staphylococcus aureus*. Nevertheless, in 1890 Koubassoff found in human cancers a short bacillus which on inoculation into laboratory animals caused disseminated nodular growths. A second report was made in 1891 by Ballance and Shattock of negative bacteriologic observations in cancer, and since that time the claims for a discovery of a specific bacterial agent have been relatively infrequent. But a number of statements have been made asserting that the cause of cancer was to be sought in higher vegetable organisms. Von Niessen in 1894, Braithwaite in 1895, Bra in 1898 and Chevalier in 1899 found mycotic organisms in cancer which were in part claimed to have a causal relationship, Chevalier believing that this view was supported by inoculation experiments, which Fabre-Domergue and Richardson were unable to confirm.

The attempted identification of saccharomycetes or blastomycetes as the cause of cancer has given rise to such an extensive literature as to necessitate its discussion as a separate item. To continue the more general aspect of the subject, a report by Monsarrat to the effect that he had recovered from cancers a pleomorphic organism that would produce in guinea-pigs tumor-like growths, on being reviewed by a committee of the Pathological Society of London, brought the verdict that these were of granulomatous character. In 1905 Hoffmann announced the finding in ulcerated cancers of spirochetes, but although this was confirmed by Loewenthal in 1906, Beebe and Gaylord in 1907, and by Deetjen and Simmonds in 1908, the significance of their relationship to the ulceration was quite generally recognized, only Gaylord suspecting that they might play a causative rôle. Deetjen, indeed, had found them present in nonulcerated tumors in the mouse, but he was able to find a strain of the same tumor in which they were absent, and to show that in some unsuccessful attempts at transfer of tumors, the spirochetes might be inoculated independently; in 1919 Mueller showed that it was possible to destroy the spirochetes in these tumors without affecting the latter.

The *Micrococcus neoformans* of Doyen—as its name indicates, a coccoid organism in which he believed that he had found the cause of the disease, with apparent production of cancers in rats following its inoculation—met with the same fate as previously announced bacterial causes. Karwacki regarded it as a secondary invader, and Letulle

reported that the alleged tumors caused by it were in reality of inflammatory character. In 1914 Minett identified this organism with *Staphylococcus albus*, which is frequently found in the deeper tissues. Much of the interest in *M. neoformans* lies in the explanation by Doyen of the mechanism of its production of tumors—the provocation by it of excessive and abnormal phagocytic activity, which, leading to intranuclear parasitism, gave the cells the property of unrestricted growth.

In 1908 Robertson reported that he found in cancer rod-shaped organisms, which, however, he regarded as a stage in the life cycle of a conjectured protozoon of alleged causal relationship. In 1921 the same writer implicated large diphtheroid organisms as the probable cause. Leyton in 1916 claimed to have isolated from rat tumor a filtrable streptothrix which on repeated injection into rats caused in them inoculable sarcomas, and in 1920 King found in cancer a fungoid organism with a complex life cycle, for which he also claimed positive results on inoculation. In 1921 Nuzum reported that in cultures from transferable mouse cancers he obtained a coccoid organism that produced tumors in inoculated mice; in 1925, with a streptococcus isolated from malignant tumors, he observed that following its inoculation into the breast duct of dogs, there was a development of cancer there in 2 of 5 attempts, 1 of the dogs dying with generalized cancerous metastases. Also in an elderly man, who had previously had an epithelioma, a similar tumor appeared after repeated inoculation with this organism. Nuzum's earlier work was checked, unsuccessfully, by Kross.

The etiologic relationship of pleomorphic bacteria to malignant tumors has been the subject of a number of claims. Young in 1921 stated that tumor developed in 10 per cent of mice inoculated with such an organism. Glover, and Loudon in confirmation of him, made similar claims, which Kolmer, Harkins and Saleeby, after repeating Glover's experiments with scrupulous observance of his technic, were unable to verify. Purpura isolated from cancer a pleomorphic organism for which, however, no etiologic relationship was suggested, and Lieske as recently as 1928 implicated such an organism as the cause. Nissle in 1927 stated that in mice he had caused cancer by the injection of molds or their filtered broth cultures; Lumiere and Montoloy, also in that year, failed in 45 tumors to find any organisms that were not almost certainly secondary invaders.

Reference has been made to the suggested identification of blastomycetes as the cause of cancer. The stimulus to this was furnished by a report by Busse to the effect that these organisms had been found to cause a human lesion which he first believed to be a giant cell sarcoma of bone, although in a later report he recognized it as a chronic inflammatory lesion. Even before this, Russell had described his "fuchsin bodies," which he had interpreted as yeasts, though Klien, Dean and

Councilman had announced that in their opinions they were, like the alleged protozoa, simply the products of degenerative changes; Bergonzini, while disposed to concede their living character, questioned any possible relationship to the cause of cancer. In 1895, the year of Busse's amended report, Sanfelice, Aievoli, Rossi-Doria, Kahane, D'Anna, Curtis, Roncali and Corselli reported the isolation of organisms of this type from tumors, and in some cases the causation by these of tumors in laboratory animals. Similar claims were subsequently made by Binaghi, Mirto, Hare and Harris, Plimmer, Leopold, Wlaeff and Fabry and Trautmann. Sanfelice believed that Russell's bodies could be identified as of the type of the saccharomycetes which he regarded as the cause of tumors, and Roncali announced a similar identification of Korotneff's parasites. But Mafucci and Sirleo, although believing many malignant tumors to be of infectious origin, recognized the inflammatory character of blastomycotic lesions, as did Frothingham, Bonome, Galeotti, Henke, Nichols and Petersen and Exner; Lubarsch and Franchetti reported negative results following the inoculation of animals; Sternberg regarded the alleged blastomycetes in tumors as degenerative products; Lecount and Greenough made the same identification of the alleged organisms described by Plimmer, and Spirlas found that Plimmer's bodies could be produced by the injection of a considerable number of diverse agents. Alessandri in 1903 reported that cultivation attempts with 33 tumors yielded consistently negative results as regards blastomycetes, although that they were occasionally present was shown by McCampbell, who recovered them from a spontaneous tumor of a wild mouse, but was unable to find any tumor-producing action by these in experimental transfer. Loeb, Moore and Fleisher only exceptionally found yeasts in malignant human tumors, and their attempts at the causation of tumors by yeasts yielded only inflammatory lesions. Of late years the attempted identification of the blastomycetes as the cause of malignant growths has been almost completely abandoned, although Pentimalli in 1912, having observed local hyperplasia of tissue in fowls into which endotoxins of these yeasts had been injected, expressed the belief that infection with them may be a contributing factor; Roncali in 1914 continued to regard them as one of a number of parasites acting as causes, and as lately as 1929 Sanfelice believed that he was able to observe amelioration of human cancers treated with preparations of blastomycetes. A reexamination of Russell's fuchsin bodies by McConnell and Lang in 1921 led them to conclude that these bodies were the result of cytoplasmic degenerative changes. There are few organisms that are more definitely associated with hyperplasia of tissue than are the pathogenic yeasts, and it was undoubtedly the irregular and excessive epithelial overgrowth which these infections occasion that led to the conclusion

that they were the cause of cancer, despite the absence of independent and unrestricted, and of remote, growth.

**Bacillus Tumefaciens:** A discovery of association between certain bacterial infections and the inception of tumors, in which the exact relations are still far from clear, resulted more or less directly from a study of crown galls in plants. A similarity between plant galls and animal tumors was noted even in 1887 by Paget, and Packard again called attention to it in 1911. In an article published in 1912, E. Smith emphasized the resemblance of crown galls to malignant tumors, especially as regards the features of invasive and secondary growth, and a few years later he was able to show that these lesions of plants were caused by infection with a bacterium which he termed *Bacillus tumefaciens*. The close analogy of the plant and animal tumors is still disputed. Levin, who in 1920 accepted the analogy, in 1922 pointed out that unlike the neoplasms of animals, those of plants possessed tissues capable of complete differentiation. Blumenthal, along with Hirschfeld, also expressed doubt of the similarity, and Stapp in 1927 expressed the belief that the crown galls were more properly to be regarded as granulomas, while Vasiliu regarded them as more analogous to benign tumors of animals. Nemec in 1928 also viewed them as granulomas, as did Scaglione and Hamdi in 1929. Agreeing with Smith in regarding them as plant cancers was Magrow in 1924, Auler in 1927 and, as will appear shortly, Blumenthal in a number of his later reports. In 1920 Smith was able to secure tumor-like hyperplasia in plants by the application of dilute alkalies and acids, by the introduction of foreign organisms and by partial asphyxiation—results which would appear to suggest a granulomatous reaction—a view further sustained by the causation in plants of lesions similar to the bacterial crown galls by the injection of 1 per cent lactic acid solution, as reported by Blumenthal and Meyer in 1923.

A year later Blumenthal, Auler and Meyer reported that from 12 of 30 human tumors they had succeeded in isolating organisms which on inoculation into animals induced malignant tumors; the latter finding was confirmed by Pickhan. Especially significant appeared to be the fact that the organisms could not be recovered from the induced tumors. In spite of certain cultural differences, it was at first believed that the several bacterial strains isolated were closely related to each other and to Smith's *B. tumefaciens*, a belief which was not substantiated by later work, especially by that of Reichert, who demonstrated decided differences in the several organisms, not only in cultural characters, but also in regard to toxicity and immunity reactions, this last fact being later confirmed by Becker. It was the usual experience that *B. tumefaciens* of plant origin was without effect on animals—among the published experiments to this effect being those of Teutschlaender and of Borghi

and Luzzatto. Those of animal origin, on the other hand, were frequently active in plants, as shown by Kauffmann with a bacillus isolated from mouse cancer, by Fejgin, Epstein and Funk, and by Bechhold and Smith—at times even when their action on animals was no longer manifest.

That the tumefacient action of these bacteria is apparently not dependent on the organism itself is indicated by a number of observations. So in 1919 Blumenthal observed that accidental contaminants at times became possessed of this effect, a result which he was not able to achieve intentionally. The outstanding differences between various organisms of the tumefacient group led Reichert to seek a common property in a filtrable virus which he believed they harbored, a view which was later shared by Blumenthal, largely on the basis of Gye's work. The finding by Kauffmann that the power of inducing tumor could be lost by organisms on cultivation, and that of Friedemann, Bendix, Hassel and Magnus that organisms apparently identical with the *B. tumefaciens* of plants, even as regards immunity reactions, but without tumefacient power, could at times be isolated from human inflammatory lesions, supported the idea that some extrabacterial agency was concerned in their action. Bechhold and Smith, indeed, found that the active agent could be filtered through a membrane holding back particles more than 20 micro-microns in diameter, could be boiled for over 20 minutes without destruction, and would resist the action of 1 per cent phenol for 1 hour. But there is no general agreement as to the more significant of these findings. Kauffmann, in his latest work, found that living organisms were necessary for the production of tumor, and regarded the tumors as granulomas. It had been emphasized by Blumenthal, Auler and Meyer that softening or ulceration of animal tumors was necessary for the recovery of the organisms, and R  th reported their rather general occurrence in animal tumors that had been subjected to a process of preliminary artificial digestion. Although his results were regarded as confirmatory by Blumenthal, R  th's later finding, that by this process he could recover Blumenthal's bacillus from the tumors that were caused by its inoculation, seems to cast doubt on the autonomous growth of those lesions, and possibly relegates them to the group of granulomas.

*Distribution of Cancer as an Evidence of Infectivity.*—Cancer Houses and Localities: There is a considerable body of accumulated literature bearing indirectly on the subject of the infectivity of cancer. In this are a number of instances of local distribution in circumstances such as to suggest the action of a common etiologic factor and hence the possibility of infection. Instances of the localized concentration of cases of cancer are rather numerous; in some cases these are associated with peculiarities of water supply or drainage, while others are without

this association. Among the former are those reported by Alderson in 1885, indicating a riverine distribution; those by Haviland in 1890 and 1895, of "cancer houses" located in regions liable to flooding, although in the later of these reports the possible action of an hereditary factor is mentioned; those by Fiessinger in 1893, who from the usual proximity of such areas to riverine or wooded regions postulated a factor of insect transmissibility; those by Webb, who in 1894 reported on cancer houses that were apparently related to water supply; those by Nason, who in 1898 reported on British registration areas where high incidence of cancer coincided with low-lying, poorly drained land, and those by Blake and by Power, of riverine cancer in England. A summarized report in the *British Medical Journal* for 1900 describes poorly drained regions in Warwickshire, Staffordshire, Shropshire and Worcestershire which had unduly high cancer rates. Mason in 1902 and Plowright in 1904 discussed similar instances, the latter finding especially high cancer death rates in waterlogged areas with surface water in the wells, with 50 per cent of the cases occurring under the 50 foot (15.2 meter) level, and only 10 per cent above the 500 foot (152.4 meter) level. Behla, in the triple community of Luckau, Kalau and Sandau, found frequent cases of cancer in the two former, low-lying settlements, and none in the last, which had a high, well drained site. Pöppelmann reported localized incidence of cancer in a German village with apparent relations to the drainage system. Other reports of similar tenor have been made by Prinzing, Kolb, Unglert, Sachs and Abramowski, the last with an account of a sandy, well drained point that was free from cancer, while on nearby marshy shores it was common; Eichhorn, in a survey of cancer distribution in the district of Constance, in southern Baden, has found this same relation to drainage. Stelys described an apparent case of relation between water supply and cancer, with inferences of causation quite unlike the usual one of infection; in Clermont the disease appeared to show localized distribution along a volcanic fissure of the subsoil, which was the site of numerous carbonated springs, an association which he suggests may be due to the excess of carbon dioxide, deficiency of oxygen or the presence of radio-activity. By no means all the instances of localized cancer concentration are linked with peculiarities of water supply. Wheeler in 1885 reported localized centers in Castine, Me., which had no such relation; Scott in 1900 could find no evidence of such a relationship in eastern Essex; Hoeber in 1904 found that in Augsburg, while cancer was more frequent in the lower portions of the city, these parts were also the oldest and most densely populated. Gordon and Thompson in 1913 found in two registration districts in Cornwall very uneven, patchy distribution of cases of cancer with, however, no apparent relation to water. Nor could Robertson find any suggestion of riverine incidence in the distribution of cancer in

Scotland. There is at least one report of the latter distribution in circumstances in which another, definitely known etiologic factor of cancer plays its part, the riverine occurrence in Kashmir of kangri cancer, as reported by McCulloch.

Other reports of the geographic concentration of cases of cancer without special relation to water supply or to drainage are those of Arnaudet, Lucas-Championniere, Guelliot, Noel, Fabre-Domergue, Loeb (in cattle), Kruse, Sticker, Tynes and Sambon. Some of these have been accompanied by suggestions as to etiology other than that of active transfer of an exciting agent. Loeb believed that the localized occurrence of frequent cases of cancer in cattle from a certain ranch in Wyoming could be best explained by heredity; Kruse ascribed the relatively high frequency of cancer in northern Italy to racial influences, and Pick, writing of the endemic occurrence of thyroid cancer in *Salmonidae*, believed that this was due to some deficiency in their water.

The reports of cancer houses are numerous. Behla, Brand, Guelliot, Fiessinger, Haviland, Lucas, Lyon, Mason, Power, Rohdenburg, Sambon and Webb have reported instances of these, in some cases of unusual interest. Rohdenburg was able in his cases to establish with some regularity, in all the cases of cancer concerned, a common factor of chronic irritation. The usual view of the so-called cancer houses is that of von Hanseemann, who regarded them as being purely the result of coincidence. Mathematical analysis is undecided as to this. Pearson, after such analysis of the distribution of cancer in one (British) registration area, believed that it showed the presence of cancer houses beyond the possibility of coincidence, and study of the individual houses to his mind excluded explanation by occupation or by heredity. McKendrick, on the other hand, after mathematical analysis concluded that the occurrence of such houses was purely fortuitous.

Cancer a Deux: The numerous cases of cancer a deux, such as those collected by Behla, Bernstein, Budd, Castueil, Levin, McEwen, Martin and von den Velden, are as difficult to interpret in terms of etiology as are the cancer houses. A few are of unusually suggestive character, as that of Martin, in which a wife developed a squamous cell carcinoma of the breast at the site where her husband, to obtain comfort, was accustomed to rest his cancerous ear. While this might be a case of implantation from one person to another, the unusually interesting case of apparent transfer reported by Lecene and Lacassagne does not admit of such an explanation: Here a medical student, in aspirating serous fluid accumulated in a cancerous breast, accidentally injected some of it into his hand, and a few months thereafter an eventually fatal sarcoma developed at the site of the wound. The mathematical probability of cancer a deux has never been satisfactorily determined. Von den Velden believed that his data, based on 1,915 cases of cancer, showed its occurrence in

7 times the probable frequency. Levin, on the other hand, observed cancer a deux in only 18 of 4,000 collected cases.

"Cage Epidemics": Occasional phenomena have been observed in laboratory animals that somewhat resemble those of cancer houses or cancer a deux. Endemic outbreaks of cancers in animals have been described by Borrel, Loeb, Michaelis, Gaylord, Haaland, Negre, Aschner, Henke, Klinger and von Wasielewski. Loeb believed that those reported by him could be best explained by hereditary factors, and while this explanation applies to the outbreak reported by Michaelis, that reported by von Wasielewski was peculiar in that it involved only 1 of 9 cages of similarly bred mice, and Klinger believed that the hereditary element could be definitely excluded in the instance reported by him. Bashford, Murray and Cramer suggested that apparent outbreaks of this sort in laboratory animals might be merely the result of the accumulation of an excessive proportion of aged individuals. In the case of the endemic occurrence of the thyroid tumors of fishes, it has been established by Marine that a large part is played by a deficiency of iodine.

*Helminth and Other Higher Animal Parasites.*—The association of malignant neoplasms with infestation by various helminth parasites, which was placed on a definite experimental basis by Fibiger in 1913, had been noted clinically for a considerable period before that. Groth in 1864, Klopsch in 1866 and von Linstow in 1868 had reported cases of carcinoma of the breast associated with infection by *Trichina*, and the second named had expressed the surmise that the one process might have a causal relationship to the other. A case of cancer of the breast with trichinosis reported by Strandgaard and one of cancer of the skin by von Langenbeck—references that I have been unable to verify—are cited by Lewin. Since these early reports, cases of this association have been infrequent, probably because of the more effective control of the parasite, although Babes reported a case of bronchial carcinoma, and Babler one of cancer of the lip, associated with trichinosis, and in 1920 Fibiger was able to cite another case, of cancer of the tongue.

*Schistosoma haematobium* as a tumefacient agent was first reported in connection with polyps of the bladder by Sonzino in 1876 and by Couenon in 1881. Belleli in 1885 observed what he regarded as this parasite—more probably it was *S. mansoni*—in association with a rectal fibro-adenoma, and in that year Kartulis reported that the tumor-like growths of the bladder were of such a character as to lead occasionally to rupture. Apparently, however, the first definitely diagnosed carcinomas associated with *S. haematobium* were those reported by Harrison in 1889. Albarran and Bernard in 1897 reported a similar case, and in 1898 Kartulis reported not only a number of cases of vesical cancer, but also one of cancer of the foot which showed relations to the parasite,



and occasional cancers of the rectum and prostate (*S. mansoni*?). The most comprehensive discussion of the relationship of *S. haematobium* to cancer is that of Goebel, who called attention to the stage of preliminary irritation induced by the parasite, as well as to the blockage of blood and lymph vessels by the ova.

In 1890 Toison reported the finding of unidentified ova in the central portion of a sarcoma, and in 1898 Kanamori described in detail the observations in a case of rectal carcinoma associated with the ova of a parasite unidentified at that time, but since recognized as *S. japonicum*. That this case was not exceptional was shown by Kazama, who in 1921 pointed out the frequency of intestinal cancer in districts infested with that parasite, and its association with the ova. Obviously tumefacient properties would appear to be generally associated with *Schistosomidae*.

*Distomidae* would appear to have almost equally great powers of inducing tumor. Askanazy in 1900 reported in East Prussia the occurrence of hepatic cancer associated with *D. felineum*, and Katsurada 2 cases of cancer of the liver among 76 cases of infestation with *D. spathulatum* studied by him. In addition to its occurrence in East Prussia, *D. felineum* has been reported as causing cancer in Russia, by Ruditzky, and in Holland, where Hoogland found 5 cases of cancer of the liver in infested cats and reported on the apparent association of *Distoma* with cancer of the liver in cattle. With this parasite, as with *S. japonicum*, the preliminary changes are those of chronic inflammation; Paul in 1927 called attention to its action in inducing cirrhotic changes in the liver.

A rather wide range of parasites have shown relationships to cancer in lower animals, in addition to those already cited in relation to human cancer. Borrel in 1906 reported on the frequency of rat sarcomas in connection with *Cysticercus*, a finding confirmed by Regaud in 1907. Bridre and Conseil and also McCoy in 1909, Woolley and Wherry in 1911 and 1912 and Bullock and Rohdenburg in 1913. Experiments at the induction of tumor with this parasite, *Taenia crassicaudes*, were made with very doubtful success by Saul in 1907, but in 1920 Bullock, Curtis and Rohdenburg were able to produce sarcomas of the liver in rats fed with the eggs of the parasite. Here, too, as they had shown in a previous report, the development of a malignant condition is preceded by changes of a chronic inflammatory character. Of miscellaneous parasites that have from time to time been connected with the appearance of tumors in animals are: *Trichodes crassicauda*, which Loewenstein reported in 1910 as causing papillary tumors of the urinary bladder in rats, occasionally with some indications of malignancy; even proximity of the ova appeared to suffice to induce the changes, which were without evidences of preceding inflammatory hyperplasia; *Rhabditis pellio*, reported in 1919 by Kopsch as causing both carcinomas and sarcomas in infested

frogs; experimental attempts at the induction of cancer by this parasite, reported by Puder in 1925, were unsuccessful. Other parasites are *Dispharagus* sp., which von Wasielewski and Wülker found associated with malignant tumors in the stomach of the dove; *Hepaticola hepatica*, which Beatti in 1923 found in connection with gastric cancer of the rat, a finding that was confirmed by Bonne in 1926 and studied experimentally by Vogel in 1929 with reported success, although the photomicrographic illustrations that accompany the report are not entirely convincing; *H. cancerogena*, a parasite regarded by Beatti as distinct from *H. hepatica*, which he found also frequently associated with gastrointestinal cancer in the rat, although feeding experiments on pied rats were unsuccessful. A number of interrelationships between parasites and tumors are less definitely established. This is particularly true of the suggested connection between acarid infestation and cancer. Borrel, who in 1906 suggested that parasites might be the carriers of a cancer virus, in 1908 and 1909 reported the frequent presence of these parasites in human facial cancers; Negre in 1910 ascribed a cage epidemic in mice to the agency of these parasites, and Saul in that year called attention to an allegedly wide distribution of acarids in human and other tumors. Among the acarids found by Saul was one identified by Dahl as a member of the genus *Tarsonemi*, acarids that in plants evoke gall formation. Ascher, also in 1910, observed in rats an acarid infestation that caused papillary proliferation and limited downward growth of the skin. Von Wasielewski, too, in 1912 found limited epithelial overgrowth in rats irritated by these parasites, and Chambers and Somerset in England in 1923 found acarid infestation frequently associated with cancer of the breast. In 1928 Sambon suggested an association might be established between cancer of the skin in mice and the presence of *Psoergetes simplex*. Du Bois, cited by Lewin, reported a case of cancer of the skin apparently having its inception at the point of lodgement of an acarid.

On the other hand, Tsunoda in 1910 found the same relative frequency of acarid infestation in normal and cancerous breasts, and Orth objected to their implication as causative agents of cancer on several grounds—the data of Tsunoda, the lack of relationship between the age incidence of the two conditions, and the fact that these parasites are especially frequent in hair follicles, which rarely become cancerous. Reuter published observations showing the readiness with which acarids could invade tissues after their removal from the body. Joest and Ernesti in 1916 could find no relationship between acarid infestation and cancer in birds, and Marsh, although he admitted the possibility of their acting as chronic irritants, found them only rarely in tumors in the mouse, and could find no experimental evidence of any relationship to cancer. Similarly Teutschlaender in 1919, although he found

that these parasites in rats caused a form of itch, could find no relationship of this to cancer in that animal. However, in 1922 he reported a cancer of the foot in a chicken locally infested by acarids. Amormino in 1929 found in rabbits that acarids caused only cutaneous inflammation.

Other parasites of somewhat doubtful association with cancer are the filariae, which were reported by Borrel in 1927 as being of frequent occurrence in the spontaneous cancers of the breast in mice, a finding that is disputed by Dobrovokskaia-Zavadskaia and Kobozieff. *Echinococcus* infestation has been suggested as an occasional cause of cancer of the liver by Ziegler. Naïck and Liang reported two carcinomas of the bile tracts in the liver, in association with the presence there of *Clonorchis sinensis*.

In spite of the frequently observed association between various parasites and cancer, this association might still be regarded as coincidental if it were not for the work of Fibiger, to which allusion has been made. Basing his work on the observation of frequent gastric cancers in rats from the warehouse district of Copenhagen, he was able to establish the fact that these were related to infestation with a parasite named by him *Spiroptera neoplastica*, and the further fact that the intermediate host of this parasite was a roach of American origin, evidently transported to the warehouses by ships. Feeding experiments with these roaches definitely showed their relation and that of the parasite to the rat cancers, and by means of them he was able to produce cancers of the stomach not only in the pied rats in which most of his experiments were conducted, but, though less frequently, in other strains of tame rats as well, and, though rarely, in mice. With the exception of the causation of malignant tumors by Marie, Clunet and Raudel-Lapointe by means of the x-ray, which was essentially the repetition in experimental conditions of a well established clinical fact, this represents the first experimental induction of cancer strictly de novo.

As to the mode of action of the animal parasites in inducing malignant growth, little is known. Their mere ability to induce chronic inflammatory changes, although, as has been shown, frequently associated with the appearance of cancer, cannot alone suffice to explain their tumefacient effects. A number of irritants that produce inflammatory changes of equal or greater degree are only exceptionally associated with the causation of cancer, as was pointed out by Fibiger in connection with infestation by *Trichodes*, a parasite of the same size, and producing direct effects similar to those of *Spiroptera*, but rarely if ever causing cancer. Borrel in a number of publications views these parasites as the carriers of a presumably living virus, and Mercier and Lebailly have suggested that a myxosarcoma of the fowl which they reported may have been caused by the transfer by acarids of a filtrable

agent of the Rous type. Saul in 1915 ascribed their action as due to metabolic products, an explanation which, as far as it goes, seems as satisfactory as any that is likely to be adduced. The finding of Flury and Leeb that the metabolism of *Distoma* is largely of anoxybiotic type, is interesting in view of the experiments on tumor metabolism of Warburg, and hints toward a possible clue in that direction.

*The Filtrable Agent.*—The discovery by Rous in 1911 of the first of a series of fowl tumors that are transferable by cell-free material introduced into the study of the etiology of tumor an element that even yet is not satisfactorily explained. Quickly following the report on this first tumor, the finding of a number of similar ones was announced by Rous, Rous and Lange, Tytler, Fujinami, Muto, Inamoto and Ogata and Ishabashi and later cases by Begg and Teutschlaender. These tumors are all alike in that they occur in fowls, and in that they may be transmitted by almost certainly cell-free filtrates, by properly prepared desiccated tumor tissue and by glycerinated extracts. They vary in morphology, but the morphologic type of the original tumor is as a rule preserved in the induced tumors, although to a certain extent, as shown by Rous and Murphy, variations in the tumor may depend on the host. In addition to the confirmation of Rous' findings as afforded by the discovery of similar tumors, his detailed observations were quickly confirmed by Walter and Bürger, and since by an enormous volume of related work. Bürger found that not only could these tumors be transmitted by the means mentioned, but also at times by the blood or by the ascitic fluid of involved animals, and even by tumor-free organs. As regards transmission by blood, Llambias and Brachetto-Brian were able to show that not only whole blood, but even washed red cells, could be effective, and Lewis and Andervont, that this was true both of plasma and washed leukocytes, the latter being the more certain. Fraenkel found that while splenic tissue, free from metastases, could cause these tumors at times when inoculation with blood gave negative results, no results could be anticipated from either unless the tumor had progressed to a considerable size, and that then even the yolk of an ovarian egg might be active.

In addition to the specificity as to type of tumor, these filtrates also show marked specificity as to animal susceptibility. With the original Rous tumor, this was at first limited to related fowls, and only later did Rous and Murphy find that the selectivity gradually disappeared, and while eventually they found that the virus was capable of inducing tumor in embryonic pigeons and ducks, this is restricted, as with the transplantation of tissues, to the earlier nonspecific stages of development.

Closely related to the action of the filtrate is the matter of injury to tissue, as was early shown by Rous and his co-workers, who found that care taken to minimize this greatly reduced the frequency of the induction of tumor. That this is possibly due to fixation of the agent by

injured or regenerating cells is suggested by the work of Pentimalli, who found that injured cells and embryonic cells possess such power of fixation, and Mackenzie and Sturm, who found the virus localized in the early stages of induced local inflammatory reactions. In the opinion of Rous, Murphy and Tytler, the metastases that are a prominent feature of these tumors seem largely to be the results of cell transplantations, as with the general run of malignant tumors, except to the extent that they might result from the action of the agent on injured tissues; however, Fischer has found that in tuberculous fowls the tubercle bacilli may be found in the primary tumor only, which would apparently indicate the induction of metastases by the cell-free agent.

As to the nature of this agent, in many respects it obviously resembles an ultramicroscopic living virus, even to the presence in the tumor cells, as shown by Borrel, of granulations like those of molluscum or vaccinia. Such was the view taken by Gye, who in 1925 published the results of experiments that appeared to indicate that it contained such a virus, capable of cultivation, which could be destroyed by the action of certain antiseptics, whereupon the filtrate became inactive until its activity was restored by the addition of other tumor extracts, even from entirely unrelated animals. In other words, the fresh filtrate of the Rous tumor contained two elements necessary for the induction of tumors—a living, nonspecific virus and a specific adjuvant, the presence of which permitted the attack of the virus, and which gave the filtrate its properties of specificity both as to host and as to type of tumor. Gye's work offered by far the most satisfying explanation of the properties of the filtrable agent and received a certain amount of confirmation by Baker and in part by Mackenzie and Illingworth and Sittenfield and Johnson. But by far the greater number of experimenters who checked Gye's work were unable to find confirmatory evidence. Even in the year of Gye's original report, Roussy expressed the doubt that chloroform, the first antiseptic used by Gye, could be relied on to destroy with certainty the supposed virus, and Cutler in 1926 and Fraenkel in 1926 and 1927 published experimental results indicating that such was the case. It was quickly shown, too, by Flu, Murphy, Sternberg and Cori, that various non-neoplastic tissues—placenta, embryonic tissue, cultures of normal organs, even foreign blood—could supply what Gye regarded as the nonspecific virus. Pentimalli in 1927 repeated Gye's work with careful observance of his technic, without being able to confirm his results, and a repetition of Gye's work undertaken by Gye and Mueller, with the use of acriflavine as an inactivator, gave results so uncertain as to be on the whole nonconfirmatory. And Kolmer and Harkins, using concentrations of chloroform and acriflavine in degree sufficient to insure inactivation, were unable to find any evidence of a restoration of tumor-producing power. Illingworth and Alexander, too, found that

inactivation by ultraviolet light did not admit of reactivation. With experiments as complicated as those of Gye, the possibilities of uncertainty are numerous. The unreliability of dilute antiseptic agencies has been mentioned. Murphy explained the positive results as due to the presence of a ferment that, after inactivation, was still capable of reactivation; Harkins, Schamberg and Kolmer believed that the alleged specific factor represented a still active, although attenuated, filtrable agent; Haaland, repeating Gye's work on the causation of mouse cancer by apparently cell-free cultures, found that the supernatant broth if entirely cell-free was incapable of producing tumors, while the cell-containing sediment could produce tumors by inoculation; in this respect, he is not in agreement with Sittenfield and Johnson, who obtained a very considerable percentage of successful inductions of tumors, by the use of rabbit serum broth cultures of mouse sarcoma, even though these had been originally inoculated with the Berkefeld filtrate of the tumor—results like those of Gye with similar tumors. Simon and Beck, who worked with an artificially induced tumor of the Rous type, believed that the occasional positive results observed in Gye's procedures were to be explained by a summation of subinfective doses or by activation of a subinfective dose; and Mueller, who in 1927 was unable to confirm not only the work of Gye, but that of Murphy and of Flu as well, believed that there were so many uncontrollable local and individual factors as to make uncertain any results that might be obtained.

A number of rather indirect efforts have been made to determine the nature of the agent. Some of these would suggest that even with the filtrate the causation of tumor is a matter of cellular transfer. Nakahara, who found that the action of the filtrate is greatly impaired by alternate freezing and thawing, and by acetone—behavior strikingly unlike that of known ferments—and who found that prolonged and excessive pulverization of the desiccated tumor would destroy its action—believed that this is due to the presence of filter-passing, minute cells, which he believed he could observe in the filtrates. This view has not been generally accepted, and in regard to the pulverization experiments, it may be pointed out that such a process would greatly enhance oxidation, which from other evidence is known to destroy rapidly the agent in question. Fraenkel believed that the possibility of cellular transmission could be absolutely excluded, basing his opinion on quantitative experiments, on the immunity of the agent to various physical and chemical agents and on the correspondence in the behavior of the agent thermally, chemically and biologically to that of the ferments. Investigation of the particulate size of the agent, which might be hoped to throw some light on the matter of possible cell transplantation, has yielded rather conflicting results. Rous and Murphy believed that its size, as determined by filtration experiments, would indicate an organized structure even to

the point of microscopic visibility; Jung found in an active de Haan filtrate formed elements that suggested the possibility of implantation; Ono found that the agent could pass through a Zsigmondy-Bachmann filter and through a Bechhold collodion membrane with a probable pore diameter of 0.6 micron, and Fraenkel, through a filter of a pore diameter of 10 micromicrons; Zinsser and Tang, using filtration through acetocollodion membranes, found for the virus a size greater than that of casein particles, smaller than that of arsenious sulphide, and comparable with that of bacteriophage or herpes virus; Baker and Peacock, who found that the agent was less susceptible to the action of ultraviolet light than are common bacteria, believed that this was due to its smaller particulate size.

Although the action of the agent is on the whole suggestive of that of the filtrable living viruses, this similarity does not hold on detailed comparison. Loewenthal found a decided difference in this respect in comparing the respective responses to capillary attraction. As concerns its powers of adsorption, the testimony is conflicting; Fraenkel, Mislowitz and Simke found that while it was adsorbed by casein, kieselguhr and kaolin, it was not taken up by animal charcoal, and in general that it showed some similarity in these respects to the filtrable viruses; Lewis and Andervont, on the other hand, failed to obtain its adsorption by kieselguhr, kaolin, india ink or charcoal, and so found a decided difference from the behavior of the viruses of vaccinia and fowlpox. A peculiarity of its behavior found by Lewis and Lewis and Andervont was that it was inactivated by a number of aluminium salts, the acetate proving to be an exception to this rather general effect; also by certain calcium salts, the composition of these being such as to suggest a negative charge for the agent—a conclusion confirmed by Murphy's and Pulcher's experiments with cataphoresis and by Pulcher's experiments on its adsorption by oxyhemoglobin. In this respect the agent probably resembles the known filtrable viruses, as that of vaccinia was shown by Douglas and Smith to have a negative charge, although Natajara and Hyde were unable to effect any separation by the cataphoresis of a number of these viruses.

While none of the data just cited are particularly decisive, striking evidence against the (living) virus character of the agent lies, as was pointed out by Murphy, in its extreme specificity; to postulate such a virus as the cause of the several tumors of the Rous type would necessitate the conception of a separate virus for each, since each is highly individual in the matter of predilection for a host, and particularly in the matter of the type of tumor that it produces.

The most conclusive evidence, however, against the theory of a living virus lies in the several reports of the causation of this type of tumor by purely artificial means. Carrel and his co-workers produced

a number of such tumors by the insertion into fowls of embryonic chick tissue, along with the injection, not necessarily at the same site, of such substances as dilute arsenious acid, indol and tar. While not nearly all such attempts have been successful—Deelman was unable to obtain Carrel's results with arsenic, and Sturm and Murphy, while they obtained a sarcoma by the injection of tar and embryonic tissue, failed to get one transmissible by filtrate—the attempts have been successful in enough cases to establish this as one method of tumor production. White and Brebner secured directly confirmatory results. To the extent that such tumors are like the spontaneous Rous tumors transmissible by filtrate, they open two possibilities as to the character of the filtrable agent: Either it is a living virus, in which case it is necessary to assume that it is harbored by a considerable proportion of normal fowls and remains latent in them throughout their lives, or it is a metabolic product which has the somewhat unusual character of being formed by the lesion that it induces. While the experiments of Carrel leave the question undecided, later experiments by Fischer, Laser and Haagen would seem to indicate the greater probability of the latter hypothesis. Fischer, and after him Haagen, reported that cultures of chick monocytes in vitro which were treated for a number of generations with dilute arsenic pentoxide gradually assumed the cultural characteristics of malignant cells, and on inoculation into adult fowls set up in them rapidly growing sarcomas, like the Rous tumors in respect to transmission by filtrate. Laser, using the plasma of a fowl that had been subjected to injections of coal tar and cultured monocytes, similarly produced a Rous sarcoma artificially. With these experiments, too, the results are not constant, as was shown by DeFries, who was unable to repeat Laser's work successfully. From the fact, however, that malignancy of the Rous type may be induced in tissue cells in vitro, in circumstances that would apparently absolutely preclude the possibility of infection, it would seem that the filtrable agent would almost certainly be of metabolic origin.

The third possibility as to the character of the agent is that it is of the nature of a ferment or enzyme. It has already been stated that Fraenkel, who found that it was separable by the methods for the isolation of ferment, considered that enough similarities exist to place the agent in this category, while Nakahara took the opposite view. Haagen, discussing the affinity of the agent for monocytes, expressed belief that it is a ferment acting largely through reduction of surface tension; Duran-Reynals and Murphy found that it could be fixed by the muscle tissue of fowls, but not by that of other animals, a behavior suggestive of that of ferments; its adsorption by injured or regenerating tissues would also be compatible with that view. Lewis and Michaelis found that the range of hydrogen ion concentration that permits action of the



agent is similar to that of bacteria, being less restricted than that of animal cells, and is compatible with that of ferments. That the agent is associated with certain protein constituents has been shown by Sugiura and Benedict, who found it in the globulin fraction; by Fraenkel, who would place it in the euglobulins, and by Murphy, who, after concentration by cataphoresis about the anode, obtained a nucleoproteid that appeared to be the agent itself. It shows a susceptibility to oxidation that could well be associated with the character of a ferment, as was shown by Murphy and by Fraenkel. Mueller showed that this oxidation could be prevented by the addition of dilute cysteine hydrochloride.

There are some, although much less definite, indications that an agency similar to that of the Rous tumor, at least in respect to filtrability, may play some part in animal tumors. A number of allegedly successful attempts at the induction of tumors by means of cancerous fluids, or cultures of such fluids, have been cited previously, but most of these have been of rather doubtful authenticity. Of more recent workers, Gye based part of his theory on his success in producing tumors in mice by the inoculation of rabbit serum potassium chloride broth cultures of mouse sarcoma, after anaerobic incubation. Sittenfield and Johnson's corroboration of these results was somewhat less successful than the work of Gye, but they were able to report the induction of 34 tumors in 105 attempts, while with the Berkefeld filtrate of the cultures they obtained only 6 tumors in 164 attempts. That the positive results could not be ascribed to implantation of surviving cells they believed could be shown by the small number of tumors that followed the introduction of the tissue itself after the same period of incubation. Using mouse carcinoma tissue, however, in similar experiments, a much smaller yield of tumors was obtained—only in 2 of 81 attempts. As already stated, similar experiments by Haaland were completely unsuccessful; Auler and Blumenthal, on the other hand, apparently succeeded in causing tumors in rats by the use of edematous fluid from human cancers, either with this alone or in combination with the cell-free filtrate of lymph glands from cancerous rats—Auler with a total of 3 successes in 16 attempts, Blumenthal with 3 in 36 attempts. In regard to these experiments, those of Sittenfield and Johnson showed a marked distinction between the results obtained with the unfiltered, and those with the filtered, culture fluids. Dealing more specifically with cell-free filtrates, a number of earlier attempts at the causation of tumors by these were completely unsuccessful, as reported by Herzog, Henke and Schwarz, Koenigsfeld and Prausnitz, Bayon, Romme and Harde, Koch, Pearce and Murphy, Harde and Henri, with tumors of rats, mice or rabbits. Morris in 3,000 mice into which the filtrates had been injected observed tumor development in only 4 of a limited number of survivors, and these in splenectomized animals in which the injection had been

accompanied by that of kieselguhr. Gaylord and Simpson, on the other hand, reported a small number of rather doubtful successes in the case of rat tumors. Rather recent work, however, of Burrows, Bisceglie and Erdmann affords evidence that such filtrates may play a part in the induction of mammalian tumors. Burrows, working with rats, found that in a small proportion of experimental animals in which had been implanted embryo tissue of the same descent, after immersion of this in the filtrate of the Jensen rat sarcoma, there was a late development of sarcoma at the site of implantation. A much greater number showed limited proliferation. Bisceglie, working with chick embryonic tissue, which was introduced into mature fowls along with the filtrate of mouse carcinoma, likewise found that this became sarcomatous, although similar attempts in mice gave negative results; in another series of experiments, it was found that if tumor filtrates were injected before the transplantation of neoplastic tissue, the malignancy of the latter was greatly enhanced. Erdmann found it possible to produce tumors of the Flexner-Jobling type in rats in which she injected a filtrate of that tumor after blockage of the reticulo-endothelial system by india ink; a few of her animals—all were of a strain very susceptible to the tumor experimented with—developed sarcomas from the filtrate alone. But results such as these, although apparently well established, are on the whole exceptional. Shattock and Dudgeon in 1916 reported that adult or fetal tissues of the mouse, implanted in adult animals after immersion in cell-free tumor extracts, failed to show tumor growth. Rous, experimenting with mixed grafts of tumor and embryonic tissue, failed to find any evidence of impartation of malignant growth to the latter. Certainly the action of these mammalian filtrates is in little degree comparable to that of the Rous type of fowl tumors, and their action appears to be only an occasional and exceptional feature of mammalian tumors. A much more constant effect is that of a temporary stimulation of growth; the limited proliferation observed by Burrows in many of his animals has been noted; Drew found that tumor extracts, made during periods of rapid tumor growth, stimulate active proliferation of cells *in vitro*—an effect that is not shown by extracts of normal organs except after autolysis; Eggers, studying the action of filtrates from a rapidly growing rat sarcoma on independently induced connective tissue growth, found with great regularity an enhancement of this, but one of temporary duration. There is some evidence that this property is common to all growing tissues, as Carnot and Terris found that material derived from fetal or regenerating tissues accelerates regeneration.

One report by Kritschewski and Rubinstein stands in apparent contradiction to the general findings just described. It deals with an alleged sarcoma of the rat, developed in response to the injection of human melanosarcomatous tissue, and transmissible by filtrate. The

findings are so exceptional in several respects, and the photomicrographs are so unconvincing as to make their identification of the lesion as neoplastic very doubtful.

**Carcinoma Sarcomatodes:** The cases of carcinoma sarcomatodes, in which there appears to be a transfer of malignancy from one cell type to another, or the occurrence of two types of malignant growth simultaneously, would suggest the action of some extracellular agent by which that transfer is effected. Quite a number of human cases of this sort have been reported—by Schlagenhauser, Herxheimer, Taylor and Teacher (6 cases), Reichmann, Lippmann, Lindemann, Leuenberger, Schiller and Schmorl (cited by Apolant), and while in some cases, as in that of Rothacker, the two malignant growths would appear to have originated independently, in many the evidence would at least suggest a transmission of malignant characteristics from one type of cell to another. There are also fairly frequent instances of similar changes in animal tumors, some observed in experimental circumstances. A number of mouse or rat tumors, originally carcinomatous, in later generations becoming sarcomatous, were reported by Ehrlich, Apolant and Haaland, Loeb, Liepmann, Haaland, Stahr, Russell, Woglom, Bashford, and Asada and Okabe. An exceptional case of this sort was reported by Lewin, in which a rat adenocarcinoma in later generations developed into a squamous cell carcinoma, a spindle cell sarcoma and a round cell sarcoma. Most usually the change is from carcinoma to sarcoma, but the reverse phenomenon has occasionally been observed—by Coenen with human material, by Lewin in rats, by Schöne in a dog, by Duschl, who observed a fibro-adenoma develop at the site of an apparently unsuccessful transplantation of rat sarcoma, and less certainly by Flexner and Jobling, who in a rat tumor originally almost wholly sarcomatous observed a later change to adenocarcinoma; but careful study of the original tumor revealed a mixed character there. Nicholson observed the development of a small epithelial tumor, apparently cancerous, in the wall of a sinus leading to an inoculated sarcoma.

It is possible that with some of these tumors the change may be more apparent than real. Asada and Okabe, in the case of the mouse tumor referred to in the foregoing paragraph, in which there was this apparent transfer of malignancy from epithelial to connective tissue, believed that the change was really only one of appearance in the epithelial cells, and Wail reported similar observations. But that this is not universally true is indicated not only by the careful morphologic studies of some of the experimental tumors of this type, but as well by such cases as that studied by Lewin, in which there is a series of changes too great to be reasonably explained by variation in cell form alone.

*Comment.*—Viewing the question of parasitism as a possible cause of cancer from the intrinsic evidence alone, without reference to the

relations of malignancy to chronic irritation, it would seem to be rather well established that all the work done in the search for a specifically causative parasite has been largely a chase after the will-o-the-wisp. None of the alleged parasites reported so hopefully has withstood the test of critical reinvestigation. It is equally well established, however, that parasitism, albeit nonspecific, may cause cancer, as with the several cancer-causing helminths. To what extent their action in inducing malignant growth is to be sought in their effects as chronic irritants, to what extent their action is even more obscure, are questions to which there is as yet no answer.

There can be little doubt that there is an agency, capable of release from the cell, which in suitable conditions is capable of inciting malignant growth. Definitely present in a number of fowl tumors, it has been studied there in some detail, although as yet the knowledge of its exact character is far from clear. The preponderating evidence would seem to indicate that it is not an independent form of life, nor in that sense a living organism; but to view it as a product of cellular metabolism, which can in turn excite the metabolic reactions that give rise to it, does little to enlighten one as to what it really is. Some hints there are that a somewhat similar agent may play a part in at least some mammalian tumors and possibly in the tumefacient bacteria; but here even the evidence of the existence of such an agent is relatively slight, and to what extent it will be found similar to the Rous type of virus can only be conjectured.

In spite of the evidence that parasites of one kind or another may cause cancer, there is little to indicate that such parasitism plays a large part in the causation of human tumors. The helminth and other gross animal parasites only occasionally play a rôle here. In regard to the tumefacient bacteria, one cannot speak so confidently. But that those organisms are to be looked at as common carriers of malignancy from one individual to another would appear to be almost entirely impossible; even their discoverers found them in less than half of the tumors they examined; their power to induce malignant growth appears to be capricious; and in spite of the numerous instances in medical literature of apparent transmission of cancer from one individual to another, those instances are the exceptions rather than the rule, and are most certainly contrary to the usual facts.

University of Nebraska College of Medicine.

## Notes and News

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**University News, Appointments, Promotions, Resignations, etc.**—John Glaister, Jr., professor of forensic medicine in the University of Egypt, has been appointed to the chair of forensic medicine in the University of Glasgow, now occupied by his father.

Sydney C. Dolruple has been appointed instructor in pathology in Tufts College, Boston.

Dudley A. Robnett has resigned as assistant professor of pathology in the University of Missouri.

Clarence G. Pfaum, instructor in pathology in the University of Minnesota, has been appointed assistant professor of pathology in the University of Missouri.

In the University of Pennsylvania Stuart Mudd has been promoted to associate professor of bacteriology.

Slikh Poloyes has been promoted to assistant professor in pathology in the Long Island College of Medicine.

The twenty-fifth anniversary of Walter B. Cannon's professorship of physiology in Harvard University was celebrated on October 15 by public exercises at which a portrait of Dr. Cannon was presented to the school.

Howard T. Karsner, professor of pathology in Western Reserve University, succeeds the late Aldred S. Warthin as member of the council on physical therapy of the American Medical Association.

In the University of Texas Ellen D. Furey has been appointed adjutant professor of pathology in place of Harry L. Klotz, and John F. Hilcher has been appointed instructor in pathology.

In the Woman's College of Philadelphia S. Brandt Rose has been appointed professor of bacteriology.

It is reported that Otto Warburg of the Kaiser Wilhelm Institute for Biology in Berlin has been awarded the Nobel Prize in medicine and physiology for 1931 for his work on the mechanism of oxidation in the living cell.

The National Research Council has given Paul E. Steiner a medical fellowship for work in pathology.

**Society News.**—The Society of American Bacteriologists will hold its annual meeting in Baltimore, Dec. 28 to 30, 1931. In the general scientific session a series of papers will be presented on "Bacterial Dissociation and Life Cycles." The topics for discussion in the section on medical bacteriology and immunology include hemolytic streptococci, meningococcus and the immunologic aspects of epidemic poliomyelitis and typhus fever.

**Commemoration of Discovery of Tubercle Bacillus.**—The fiftieth anniversary of the discovery of the tubercle bacillus by Robert Koch, March 24, 1882, will be commemorated by suitable ceremonies in Berlin from May 17 to 20, 1932.

**Medical Fellowship Board, National Research Council, Meets in March.**—The next meeting of the medical fellowship board of the National Research Council will be held in the latter part of March, 1932, and applications to receive consideration at that time should be filed not later than February 15 next.

**The Biological Photographic Association.**—An organization by this name has been formed to promote the use of photography in its various forms as aid to biologic research and medical observation and record. The president is Ralph P. Creer, director of photography in the school of medicine of Yale University, and the secretary is Theodore Nelcey of the same school.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

EXPERIMENTAL NEPHROTIC EDEMA. LOUIS LEITER, Arch. Int. Med. 48:1, 1931.

Edema has been regularly produced in dogs by adequate plasmapheresis and the resulting reduction in the plasma proteins. The critical level of edema of the plasma proteins is about 3 per cent. An interruption of the depletion of the plasma is followed by an immediate increase in the plasma proteins and an equally rapid disappearance of the edema by diuresis. The disturbance in the water balance seems to be largely a temporary upset in the normal equilibrium between the capillary hydrostatic or filtration forces and the plasma protein osmotic or reabsorption forces. It is not primarily dependent on cardiac, renal or capillary permeability factors. This experimental edema is nephrotic, as demonstrated, for the first time, by actual quantitative analysis of the protein content of the transudates. The low protein content lies exactly within the range of that of the edema fluids of clinical nephrosis and is essentially of the same order of magnitude as the concentration of protein in normal cerebrospinal fluid and of that in other ultrafiltrates of the plasma. The study of the regeneration of the plasma proteins in the depleted dogs has confirmed the earlier work of Whipple and his associates, which showed that globulin was reformed faster than albumin. The blood cholesterol tended to decrease during vigorous plasmapheresis. With cessation of bleeding, the cholesterol usually rose, occasionally to values considerably above normal. This was, however, only a temporary phenomenon and was in no way suggestive of the hypercholesteremia of the nephrotic syndrome in man. Histologic study of the kidneys in these experiments has failed to produce any clearcut evidence to support the contention of Barker and Kirk that hypoproteinemia leads to contracted kidneys. In fact, the observations in the control experiments and other facts reported seem to lead directly to the conclusion that Barker and Kirk have erred in two directions: First, they have overlooked the possibility and the frequency of "spontaneous" contracted kidneys in dogs. Second, they have apparently failed to realize the lack of essential resemblance between the so-called renal lesions of plasmapheresis and the histologic changes in the kidneys of patients with so-called nephrosis. In view of this confusion, it becomes evident that experimental nephrotic edema in dogs is not quite the same as "experimental nephrosis." Experimental nephrosis has not yet been produced. The rôle of starvation, of the administration of salt and water by stomach tube and of certain other factors in the experimental procedure has been controlled to a certain degree. The relation between experimental nephrotic edema and other experimental and clinical edemas has been discussed. In collaboration with Dr. I. S. Falk, we have presented the first published data on the protein content of the plasma and of the edema fluid in a "spontaneous" nephrotic edema occurring in a monkey.

AUTHOR'S SUMMARY.

NEPHROSCLEROSIS. PHILLIP F. SHAPIRO, Arch. Int. Med. 48:199, 1931.

There is no fundamental difference in the pathogenesis of benign and malignant nephrosclerosis, whatever their respective etiologies may be. Both depend, however, not on glomerular ischemia or on arteriolar occlusion, but on a hyperemia associated with a retardation of flow. This conclusion was suggested by a histologic study, checked by a series of experimental injections. The retardation is based on a neurogenic dissociation in reaction between arterial constriction and peripheral dilatation. The changes that follow are analyzed on the basis of

Ricker's views on renal hemodynamics. Moderate retardation leads slowly to the organic changes of benign nephrosclerosis. Severe retardation rapidly induces the pathologic changes of malignant nephrosclerosis and terminates in uremia. Malignant nephrosclerosis is therefore accepted simply as an "atherosclerosis renum accelerata gravis."

AUTHOR'S SUMMARY.

THE RÔLE OF THE PYLORIC SPHINCTER IN THE CONTROL OF GASTRIC ACIDITY.  
R. ELMAN and A. P. ROWLETTE, Arch. Surg. **22**:426, 1931.

When the pyloric sphincter is sectioned, a fall in gastric acidity follows. There is also an increase in emptying time. This would indicate that the pylorus exercises control over gastric acidity by controlling the reflux.

N. ENZER.

GALLBLADDER FUNCTION. I. S. RAVDIN and J. L. MORRISON, Arch. Surg. **22**:810, 1931.

The authors detected rhythmic contractions of the gallbladder in the dog, guinea-pig and monkey. On one occasion, contractions of the human gallbladder were noted. Some of the constituents of bile are found in the pericholecystic lymphatics, but not all of the constituents. The conclusion is that concentrated bile leaves the gallbladder by the cystic duct.

N. ENZER.

THE EFFECT OF SUPRANORMAL TEMPERATURES ON TISSUE CULTURES. G. PINCUS and A. FISCHER, J. Exper. Med. **54**:323, 1931.

In a study of the growth of chicken osteoblasts in cultures exposed to supra-normal temperatures of 42, 44, 47, 50 and 52 C., it has been observed that there is no lethal effect after prolonged exposures at 42 and 44 C. Cultures are killed after an exposure of 105 minutes at 47 C., of 6 minutes at 50 C. and of 3.5 minutes at 52 C. A definite inhibition of growth occurs after different exposures at all temperatures from 44 C. onward. There is a latent period of approximately 24 hours before any effect of sublethal or just lethal exposures becomes discernible; this latent period appears to be independent of the duration of the sublethal exposure and of the temperature. The high temperature coefficients for lethal exposures and for exposures just sufficient to inhibit growth indicate an underlying "destructive" process in the cells of the culture.

AUTHORS' SUMMARY.

IS THE RAT DERMATITIS CONSEQUENT ON VITAMIN B<sub>2</sub>(G) DEFICIENCY TRUE PELLAGRA? S. S. GURIN and W. H. EDDY, J. Exper. Med. **54**:421, 1931.

Isolated cases of dermatitis resembling histologically that of human pellagra have occurred in rats supplied with sufficient vitamin B<sub>2</sub>(G) in the form of beef extract or neutral autoclaved yeast to produce good growth. In other rats on basal diets containing similarly prepared nutrients, but deprived of any known source of vitamin B<sub>2</sub>(G), a dermatitis develops similar in appearance to that described by other workers, but this skin effect differs in the histologic picture from that found in human pellagra or that found in black tongue of dogs. These rats showed failure of growth, which supports the view that they lacked growth-promoting vitamin B<sub>2</sub>(G). It is suggested that dermatitis in rats may be of diverse types: one resulting from vitamin B<sub>2</sub>(G) deficiency and quite different histologically from human pellagra, and one closely allied to human pellagra and black tongue of dogs due to lack of some at present unidentified factor.

AUTHORS' SUMMARY.

THE LIPOLYTIC ACTIVITY OF TISSUES OF TUBERCULOUS GUINEA-PIGS. F. C. HAPPOLD and A. TAYLOR. *Brit. J. Exper. Path.* **12**:272, 1931.

The lipolytic activity of the liver, lung and kidney tissues of twenty-six normal guinea-pigs was studied. There was no pronounced difference between the sexes. A further series of twenty-four animals that were kept in the dark, one-half receiving ultraviolet radiation from a mercury vapor lamp, were similarly studied. No progressive change was apparent in either group as compared with the normal. A series of forty guinea-pigs were inoculated with suspensions from the same strain of tubercle bacilli. Twenty-eight were infected at the first attempt, and nine showed no sign of disease post mortem. The infected animals were killed at different stages of the disease. There was a definite sustained decrease in the lipolytic activity of the kidney tissues as the disease spread. In the liver there was an early pronounced drop in lipolytic activity, a partial recovery during the period of early tubercle formation in the organ, and a final sinking away. The extent of this late decrease was probably more apparent than real. The lung tissues, however, showed a definite progressive increase in lipolytic activity, which still revealed an upward tendency during the period of early pulmonary involvement.

AUTHORS' SUMMARY.

THE SIGNIFICANCE OF TISSUE CULTURE FOR PATHOLOGIC PHYSIOLOGY. A. FISCHER, *Verhandl. d. deutsch. path. Gesellsch.* **26**:67, 1931.

Tissue culture permits one to study the functions of the living cells in vitro. By the isolation and cultivation of cells that constitute diseased tissue the various disease processes may be better understood than by histologic methods.

In the study of infectious diseases, tissue culture is extremely valuable for analyzing the reaction of cells and body fluids against bacteria. More important still is the possibility of cultivating ultraviable virus together with living cells. Many viruses are not living organisms, but substances produced by tissue cells or blood cells; it is apparently impossible to cultivate a virus in dead material. Carrel cultures of chick embryo tissue, when inoculated with from 25 to 250 units of the virus of variola, will yield from 10,000 to 100,000 units in one week. The virus of Rous' chicken sarcoma can be cultivated for an unlimited period of time with embryonic chicken tissue. The cultivation of virus in pure cultures of tissue cells will open a new field for the study of the nature of viruses, whether they are micro-organisms or organic substances.

After the microscopic, bacteriologic and experimental era of the study of cancer, tissue culture is at present the most promising method of approaching the unsolved problems. The existence of a specific cancer cell is proved by the tissue culture, since cells isolated from different carcinomas and sarcomas and cultivated in pure cultures preserve their malignant properties and produce malignant tumors after transplantation into animals. In carcinoma as well as in sarcoma the malignancy is dependent on one cell type and there is no proof that other cells (macrophages) are necessary for the activation of the malignant principle.

Several characteristics of cancer cells in vitro help to explain the unlimited proliferation of malignant cells in the body. Cancer cells proliferate well in pure plasma or serum, while normal cells cease to grow under these conditions. The growth of malignant cells is activated when normal tissue cells are added to the culture. The normal tissue is invaded in vitro by the cancer cells and finally is destroyed. Under optimal conditions, cancer tissue grows more slowly than normal tissue, but the incidence of mitoses in cultures of carcinoma is ten times higher than in the culture of normal cells. This is explained by the fact that the average duration of life of cancer cells is extremely short. The death of cancer cells is compensated by increased proliferation of adjacent cells. The mechanism of tumor growth seems to be the same as that of regeneration. By simple mechanical lesions, a culture of normal cells can be stimulated to abundant growth, even if embryonic extract is omitted. The greater vulnerability of cancer



cells can be easily demonstrated *in vitro*; the cause of it is, however, unknown. The unlimited proliferation of tumor cells in the body is regarded by Fischer as a physiologic sequel of the vulnerability and the short life of cancer cells, and it resembles the regenerative processes following mechanical injuries of normal cells in tissue cultures.

C. ALEXANDER HELLWIG.

INFLUENCE OF CHEMICAL SUBSTANCES ON CELLS IN TISSUE CULTURE. F. DEMUTH, *Verhandl. d. deutsch. path. Gesellsch.* **26:95**, 1931.

Different chemical substances were added to tissue cultures in isotonic solutions, and their influence on the size of the cells and the nuclei and on the intensity of the growth was studied. More than 7,000 cells were measured. Calcium chloride does not change the size of the culture area, but causes a marked decrease in the size of the cells and a lesser decrease in that of the nucleus. Sodium bicarbonate, when added to calcium chloride, inhibits the calcium effect, while carbon dioxide increases it. Hydrogen ions decrease the size of the cells and that of the nuclei, while hydroxyl ions enlarge the size of the cells and enlarge less that of the nuclei. The hydroxyl ions inhibit the growth of normal, but not that of carcinomatous, tissues. Monosodium acid phosphate reduces the size of the nuclei and that of the cells. Disodium acid phosphate enlarges the normal cells, but decreases the size of carcinoma cells. Potassium enlarges only the cancer cells and inhibits the growth of normal and malignant epithelial cells. Sodium bicarbonate interferes with the growth of carcinoma tissue and enlarges carcinoma cells. Magnesium causes changes only in the cell structure. Lactic acid is without any noticeable effect. Dextrose in higher concentration is detrimental to normal tissue, but stimulates the growth of carcinoma tissue and enlarges cancer cells. Collargol decreases the size of fibroblasts, but not that of epithelial cells. Copper sulphate reduces the sizes of all kinds of cells, but inhibits the growth only of carcinoma tissue under certain conditions. Medium concentrations of heavy metals are often more effective than higher ones.

C. ALEXANDER HELLWIG.

EXPERIMENTAL ERGOSTEROL POISONING. M. HAENDEL and J. MALET, *Virchows Arch. f. path. Anat.* **276:1**, 1930.

The administration of irradiated ergosterol to rabbits and guinea-pigs caused cachexia that was associated with cholesteremia and with lipoid infiltration of the organs. The tissue changes were more marked in castrated than in normal animals. The toxic action of irradiated ergosterol is the same as that of cholesterol.

W. SAPHIR.

### Pathologic Anatomy

THE DEGENERATION OF ARTICULAR CARTILAGE IN BOVINE JOINTS. G. A. BENNETT and W. BAUER, *Am. J. Path.* **7:399**, 1931.

Constant differences in the synovial fluid of the carpometacarpal and astragalotibial articulations of the cow have been described in a previous publication. The finding of areas of degeneration in the articular cartilages of the carpometacarpal articulations of all cattle over 2 years of age would appear to be an adequate explanation of these differences. These areas of progressive degeneration in articular cartilage have been studied systematically, and the successive changes have been described and illustrated. The development of the carpometacarpal articulations was studied in a series of bovine embryos and calves. The vascular articular cartilages of embryos and calves became avascular before the animals attained the age of 2 years. Pronounced rearrangement of the subchondral bone trabeculae resulted in a relatively deficient bony support of the medial articular cartilage where the degenerative lesions occur. The possible etiologic factors of

such lesions of cartilage have been discussed. It was concluded that they were probably due to repeated trauma in weakly constructed articulations. Deficient subchondral bone support was thought to be an important predisposing factor. The type of lesion of cartilage described in this paper is not wholly similar to any of the joint lesions described in human arthritis.

AUTHORS' SUMMARY.

THE INDUCED DEVELOPMENT AND HISTOGENESIS OF PLASMA CELLS. F. R. MILLER, J. Exper. Med. **54**:333, 1931.

As the result of finding numerous plasma cells in the omenta of rabbits that had received injections of tuberculo-protein, a method to induce the production of large numbers of these cells has been discovered. The tissues in which they were pronouncedly increased were the subserosal connective tissues of the omentum, body wall and intestinal wall. The precursor of the plasma cells is a primitive connective tissue cell. As this cell develops into the typical Marshalkó plasma cell there is a progressive increase in the basophilia of the cytoplasm, the nucleus becomes eccentric, a condensation of the chromatin occurs near the nuclear membrane, and there is a loss of the nucleoli. At the time when the nucleus assumes the eccentric position, the clear area appears in the center of the cytoplasm. The early cells are capable of reproducing themselves by mitosis, while the typical mature cells divide by amitosis. The mature plasma cells often have muddy, spongy cytoplasm, which contains acidophilic or hyaline granules, as the cells grow old or begin to degenerate. The cells with granules or hyaline bodies usually have pyknotic or fragmented nuclei. These cells are the final stage reached by some plasma cells. Others, when degenerating, show vacuoles and signs of senility. Those with the granules and hyaline bodies are the so-called Russell body cells. Plasma cells developed in greatest numbers after the largest injections of tuberculo-protein. The differentiation into young, mature and senile forms was most clearly recognizable when some days had been allowed to elapse after the last large injection of the stimulating agent. A description of the plasma cell as viewed supravitaly has been given. The cells are met with in the blood stream as well as in the tissues. They are characterized by their deep yellowish-gray cytoplasm, indistinct, eccentrically placed nuclei and large numbers of mitochondria. The plasma cells differ from lymphocytes, in that they do not develop in large numbers after direct stimulation of the lymph nodes with tuberculo-protein. The young plasma cells also differ in morphology from the young lymphocytes. When plasma cells were found in the lymph nodes, they were in the connective tissue cords. The plasma cell is a definite entity, having a maturation cycle. It is stimulated to great proliferation by certain toxic irritants.

AUTHOR'S SUMMARY.

THE HISTOPATHOLOGY OF NUTRITIONAL ENCEPHALOMALACIA OF CHICKS. A. WOLF and A. M. PAPPENHEIMER, J. Exper. Med. **54**:399, 1931.

Whatever may be the nature of the nutritive error or deficiency that in chicks is responsible for encephalomalacia, the immediate cause must be looked for in some agent or condition that impairs the capillary circulation of the brain. The essential lesion is an ischemic necrosis, followed, if the animal survives, by reparative organization of the dead tissue.

AUTHORS' SUMMARY.

THE MORPHOLOGY OF THE SO-CALLED ARGENTAFFINE CELLS IN THE PANCREAS. J. M. LASOWSKY, Frankfurt. Ztschr. f. Path. **41**:1, 1931.

This article deals with the demonstration of argentaffine cells in the pancreas of the dog under various conditions. The argentaffine cells show no affinity to chromic salts. The pancreas of the normal animal always contains argentaffine cells, which under normal circumstances do not vary in number. Secretin and pilocarpine, however, injected into dogs intravenously and intramuscularly respectively, led to an increase of the argentaffine cells. Because of the similarity of

the secreting cells within the pancreas and the argentaffine cells, it is possible that they are related genetically. In atrophic conditions of the pancreas, the number of the argentaffine cells is markedly increased, owing to a transformation of the atrophic parenchymal cells into argentaffine cells. Because of the facts that the atrophic gland showed no islands of Langerhans and that there was no change in the carbohydrate metabolism, the author assumes that the dying cells of the islands are compensated for by argentaffine cells.

OTTO SAPHIR.

LIPOID CELL HYPERPLASIA OF THE SPLEEN. H. WILLE-BAUMKAUFF, Frankfurt. Ztschr. f. Path. 41:14, 1931.

Lipoid cell hyperplasia of the spleen is found in cases of marked jaundice due to compression or occlusion of the common duct, as well as in cases of diabetic lipemia and in arteriosclerosis. The principal cause of the changes in the spleen is an increase of lipoids in the circulating blood. The storage of lipoid substance occurs to the greatest extent within the spleen. Some authors believe that there is also a moderate involvement of the reticulum cells in the bone marrow and the mesenteric lymph nodes and of the Kupffer cells in the liver. Cases in which an enlargement of the spleen and marked morphologic changes in the organ are shown must be differentiated from those in which this organ contains only a moderate number of fat-containing or lipoid-containing cells. One should distinguish, therefore, between (1) cases in which there are only a few lipoid cells in the spleen (probably a frequent finding); (2) those in which there is lipoid cell hyperplasia (W. H. Schultze) and sometimes a large splenic tumor, and (3) cases of Niemann-Pick's disease and Gaucher's disease.

AUTHOR'S SUMMARY.

THE PATHOLOGIC ANATOMY OF GAUCHER'S DISEASE. A. ANTONOW, Frankfurt. Ztschr. f. Path. 41:26, 1931.

Eight cases of Gaucher's disease are reported. The opinion is expressed that the Gaucher cells arise from the reticulum cells in the spleen, lymph node and bone marrow and from the adventitial elements of the fine blood vessels within the liver and lymph nodes. The Gaucher cells in the liver are found at first in the region surrounding the central vein and later in the periphery of the hepatic lobule. It is not impossible that Gaucher cells in the liver may also be derived from the hepatic cells. Young forms of Gaucher cells in the bone marrow have an iron-containing pigment, which later disappears. Gaucher's disease is an expression of a metabolic disorder combined with a storage of a special cerebroside-cerassin within the adventitial cells of the spleen, liver, lymph nodes and bone marrow. The author believes that the occurrence of Gaucher cells not only in these organs, but also in other organs, such as the tonsils, thymus, lung, suprarenals, etc., is proved.

OTTO SAPHIR.

THE PATHOLOGIC ANATOMY OF MULTILOCULAR ECHINOCOCCUS CYST IN THE LIVER. A. POSSELT, Frankfurt. Ztschr. f. Path. 41:45, 1931.

In a large series of cases, the gross morbid anatomy of the multilocular echinococcus cyst in the liver is described. The author believes that the term "multilocularis" gives an erroneous conception, and prefers the term "alveolaris." The paper goes into many detailed descriptions which cannot be covered by abstract.

OTTO SAPHIR.

THE CHANGES OF THE ARTERIES IN INCREASING AGE. A. M. TROITZKAJA-ANDREEVA, Frankfurt. Ztschr. f. Path. 41:120, 1931.

The arteries of the elastic type reveal very early many coarse collagenous fibers. These are found in the media, are circular in distribution, and show anastomoses.

With increasing age, they become thicker and also more numerous. The arteries of the muscular type show, at first, only a few fine collagenous fibers between the muscle elements; later, however, the fibers become thicker and increase in number. The arteries of the elastic type reveal a decrease in the muscle elements in old age. Through the increase of the collagenous fibers, the regular arrangement of the muscle fibers is disturbed. The collagenous fibers in both types of arteries become indistinctly stained with increasing age. Their outlines are vague. This change is spoken of as "hyalinosis" (Voigt). There is no relation between arteriosclerotic intimal changes and the proliferation of collagenous fibers in the media. On account of the lack of clinical data, nothing can be said as to a possible relationship between the changes described in the arteries and changes in the blood pressure.

OTTO SAPHIR.

### Pathologic Chemistry and Physics

CHEMISTRY OF THE BLOOD IN THROMBO-ANGIITIS OBLITERANS (BUERGER). MAE FRIEDLANDER and SAMUEL SILBERT, Arch. Int. Med. 48:500, 1931.

A chemical analysis of the blood of forty patients with thrombo-angiitis obliterans was made to determine the total ash, total protein, calcium, phosphorus, chloride, sugar and cholesterol content. Increases in the total ash, total protein, calcium and cholesterol content were noted. No striking abnormalities were found in the values determined for chlorides and sugar. Tests for tolerance of sugar gave normal results. The conclusion that there is a tendency to concentration of the blood in thrombo-angiitis obliterans seems warranted.

AUTHORS' SUMMARY.

PROTEOLYTIC ACTIVITY OF THE SPLEEN. A. STRAUSS, Beitr. z. path. Anat. u. z. allg. Path. 85:251, 1930.

The proteolytic activity of spleen pulp was determined by subjecting Loeffler blood serum plates to the action of the pulp placed on the surface of the coagulated serum with a standard loop. A relative quantitative estimate of the proteolytic activity was obtained by noting the time at which evidence of digestion could first be detected and the depth of the digested area. The reaction optima were found to be  $p_H$  8 and  $p_H$  5, proteolysis being somewhat more active at the more alkaline reaction. Splenic tissue was lytic also for coagulated egg white and gelatin. Proteolytic activity varies somewhat with the time after death at which the spleen is removed. It begins to increase about two hours after death and reaches a maximum from one to three days after death. The maximum activity is dependent on the initial enzyme content of the tissue at the time of death, rather than on the time after death. The object of the study was to find an explanation of the softening that occurs in the enlarged spleen of acute infections, and that is sometimes seen in the enlarged spleen following acute hemorrhage. The proteolytic activity of the spleen lies at the upper limit of normal in the early stages of septic swelling and reaches its maximum in the fully developed acute splenic tumor. It is highest just before the softening of the swollen spleen occurs. The softening is therefore apparently due to autolysis of the pulp by proteolytic enzymes. As the splenic tissue again becomes more firm, the proteolytic activity of the tissue returns to normal limits. The swollen posthemorrhagic spleen is also more proteolytic than normal, but the increase is not as great as in the septic spleen. The tissue of the spleens of children from 3 to 5 years of age and of the indurated and atrophic adult spleen exhibited no increase in proteolysis, suggesting that the structure of the spleen is a factor in the process. Proteolytic activity is ascribed to the enzymes derived from leukocytes contained in the splenic pulp. These enzymes are activated by the blood serum and perhaps also by autolytic and bacterial products. That leukocytes are necessary is established by the fact that the spleens of animals and of children, which contain few or no leukocytes,

and the spleen in a case of agranulocytosis had little proteolytic activity. Absence of proteolysis in the spleen in myeloid leukemia is cited as evidence for the necessity of activation of the leukocytic enzymes. Failure of the spleen to enlarge and soften in acute peritonitis is ascribed to a possible filtrative action of the liver.

O. T. SCHULTZ.

THE OCCURRENCE OF INSULIN IN URINE. K. UEBERRACK and T. ZELL, *Biochem. Ztschr.* **239**:42, 1931.

Insulin added to urine can be demonstrated in experiments. The urine of insulin-resistant patients and of diabetic patients given too much insulin does not contain insulin.

WILHELM C. HUEPER.

COPPER IN HUMAN AND BOVINE MILK. S. G. ZONDEK and M. BANDMANN, *Klin. Wchnschr.* **10**:1528, 1931.

The copper content of woman's milk is on the average three times as great as that of cow's milk (from 0.5 to 0.6 mg. as contrasted with from 0.15 to 0.2 mg. per liter). Formula mixtures of cow's milk for infants are also low in copper content and differ little in this respect from the undiluted cow's milk. The difference in copper content in cow's and woman's milk is about 0.4 mg. per liter. In formula feeding, copper should be added.

AUTHORS' SUMMARY.

DETERMINATION OF NONPROTEIN NITROGEN BY DIRECT NESSLERIZATION. A. LUBLIN, *Ztschr. f. phys. Chem.* **200**:1, 1931.

The turbidity sometimes appearing after the addition of Nessler's reagent to the ashed material (Folin's method) is due to a reaction of the reagent with copper sulphate and the sodium tungstate used for the deproteinization. As this turbidity interferes with the colorimetric reading, the following modification in procedure is recommended: The copper sulphate may be replaced by a few drops of hydrogen dioxide and the Folin-Wu reagent by 20 per cent trichloroacetic acid, which latter can also be used for oxalated blood (from 1.5 to 5 cc.). For the ammonium sulphate solution used as standard, a urea standard solution is substituted. The urea solution, however, must also be ashed. About ten minutes are required for the determination of nonprotein nitrogen with this method.

WILLIAM C. HUEPER.

OXALIC ACID IN BLOOD. W. MEY and S. MANGERI, *Ztschr. f. phys. Chem.* **200**:31, 1931.

A method for the determination of oxalic acid in blood is described. The error range of the method is 4 per cent. Human blood contains 3 mg. per hundred cubic centimeters oxalic acid; rabbits and horse blood, from 6 to 9 mg. per hundred cubic centimeters.

WILHELM C. HUEPER.

NONPROTEIN SULPHUR IN BLOOD, PLASMA AND SERUM AND GLUTATHIONE SULPHUR IN BLOOD. J. ST. LORANT, N. HAJDIS and W. WEIL, *Ztschr. f. phys. Chem.* **200**:121, 1931.

Tunnicliffe's method for the determination of glutathione is unreliable. Therefore it was attempted to estimate glutathione indirectly through determinations of sulphur in total blood, plasma and serum. The sulphur content of plasma or of serum is practically constant in healthy persons (2.5 mg. per hundred cubic centimeters). Caro's reaction was used. In total blood, the sulphur content ranges between 6.11 and 7.30 mg. per hundred cubic centimeters. Glutathione sulphur is calculated from these figures to be between 5.42 and 4.30 mg. per hundred cubic centimeters in men and between 5.30 and 4.30 mg. per hundred cubic centimeters in women or an average of 4.86 and 4.80 mg. per hundred cubic centimeters, respectively.

WILHELM C. HUEPER.

## Microbiology and Parasitology

TUBERCLE BACILLI IN CHILDREN WITH ERYTHEMA NODOSUM. ARVID WALLGREN, *Am. J. Dis. Child.* **41**:816, 1931.

Children with erythema nodosum who give positive tuberculin reactions often excrete tubercle bacilli. This proves that the enlarged hilar shadows in the children giving the positive reactions are of tuberculous etiology, and that the children really are suffering from tuberculosis; this ought to be regarded as an argument in favor of the tuberculous nature of the erythema. At the same time, these researches yield the valuable information that children with erythema nodosum are often spreaders of infection, and that other children ought to be protected from contamination by them. Children with erythema nodosum should not go to school for a certain length of time after the disease has developed, and if they are admitted to a hospital they should be isolated.

AUTHOR'S SUMMARY.

MENINGITIS DUE TO *BACILLUS ACIDI-LACTICI* IN A NEW-BORN INFANT. HARRY D. PASACHOFF, *Am. J. Dis. Child.* **41**:862, 1931.

A case of meningitis caused by *Bacillus acidi-lactici* in a new-born infant is reported. A review of the literature shows that only three cases due to this organism have previously been reported, two in new-born infants and one in an adult. Some of the possible modes of entry of the infection are considered.

AUTHOR'S SUMMARY.

THE BLOOD PICTURE IN WHOOPING COUGH. L. W. SAUER and L. HAMBRECHT, *Am. J. Dis. Child.* **41**:1327, 1931.

After initial and terminal leukopenia had been noted in the pertussis cycle, the protocols of our experimental pertussis in young monkeys were examined. Five of the seven animals in which paroxysmal coughs developed showed an initial leukopenia, and three of the four that were not killed for histologic study showed a terminal leukopenia. This evidence is further proof that the coughs of the animals were due to infection with *Bacillus pertussis*. The blood picture is seldom an aid in early diagnosis. Initial leukopenia and terminal leukopenia are probably integral parts of the blood picture in pertussis. Leukocytosis and lymphocytosis are usually present when the paroxysmal stage is well established.

AUTHORS' SUMMARY.

A CHEMICAL VIEW OF THE PATHOGENESIS OF TUBERCULOSIS. ESMOND R. LONG, *Am. Rev. Tuberc.* **22**:467, 1930.

The first element in the pathogenesis of tuberculosis is the capacity of the bacillus to multiply in the physical and chemical environments of the tissues of the host. The growth requirements of the tubercle bacillus in artificial culture are well known. Its water relations, respiration and dependence on glycerol are distinctive. In general, animal tissues are well adapted to meet these requirements of growth. The reactions and the carbon dioxide production of the tissues may reach an inhibiting degree. The availability of glycerol may be a factor. Modifying the glycerol content of tissues artificially has a profound effect on growth in experimental tuberculosis. Growth in the animal body is usually intracellular. Its rapidity depends on the animal. Intense initial cellular response does not tend to check subsequent progression of the disease. Native immunity in man or animals appears to depend on the capacity of tubercle bacilli to multiply in the cells of the host infected. Acquired immunity probably depends on a similar factor, and is usually associated with hypersensitiveness. The latter feature profoundly affects the anatomic character. The manifestations of hypersensitiveness are a

response to the proteins of the tubercle bacillus. The necrosis of the tubercle and the acute cellular responses of tuberculosis are largely protein effects. The lipoids are responsible for much of the cellular response in its initial and chronic manifestations.

H. J. CORPER.

ANATOMICAL CHARACTERISTICS OF TUBERCULOSIS IN JAMAICA. EUGENE L. OPIE, *Am. Rev. Tuberc.* **22**:613, 1930.

A small group of autopsies made on persons of the Negro race in Jamaica are described because they furnish anatomic evidence of the acute character of the pulmonary tuberculosis prevalent in Jamaica. Observations made on dispensary patients showed that the average duration of the disease in Jamaica, as elsewhere, varies with the age at death.

H. J. CORPER.

THE EFFECT ON GROWTH OF THE TUBERCLE BACILLUS OF MODIFICATION OF THE AMOUNT OF GLYCEROL IN THE TISSUES. ESMOND R. LONG and ARTHUR J. VORWALD, *Am. Rev. Tuberc.* **22**:636, 1930.

An attempt was made to apply the knowledge of the nutrition of the tubercle bacillus in laboratory culture to the growth of the micro-organism and the spread of tuberculosis in animals. Glycerol, fed or injected over long periods of time into tuberculous rats, appeared definitely to enhance the growth of tubercle bacilli in lesions, and to increase the extent of the disease, reproducing to some extent in vivo the conditions of growth in vitro. In the attempt to produce the opposite effect, a reduction of the amount of free glycerol available for bacillary growth in animal tissues, palmitic acid was administered in the largest amounts tolerated by the animal of the experiment, the theory being that an excess of fatty acid in the body would force a synthesis of some of the free glycerol present with the fatty acid administered, to form neutral fat, which does not support the growth of the tubercle bacillus. The results were not consistent, but in the majority of cases the extent of tuberculosis was less in the animals treated with palmitic acid than in the controls. The fatty acid was deleterious to the general health of the animals treated. An unexplained finding was an increase in the number of visible acid-fast bacilli in the animals treated with palmitic acid, in spite of a decrease in the extent of the tuberculosis.

H. J. CORPER.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN. FLORENCE B. SEIBERT and BETTY MUNDAY, *Am. Rev. Tuberc.* **23**:23, 1931.

The tuberculin action of tuberculin protein, as seen, for example, in the skin reaction in tuberculous animals, appears to be due to a specific portion of the protein molecule, which seems to be present in the first, but not the subsequent, cleavage products obtained during hydrolysis of the protein. The smallest protein molecule in tuberculin capable of giving the reaction has probably a comparatively low molecular weight, of from 1,000 to 2,400 or less, as indicated by passage through membranes. Only the protein and possibly its first cleavage products produce the precipitin reaction in serums from animals immunized with the protein-carbohydrate complex (that is, the whole ammonium sulphate precipitate of tuberculin prepared from the human type of tubercle bacillus). The lethal power of the tuberculin in tuberculous animals and in normal animals is probably associated with the very early cleavage products of the protein. Polysaccharide is chemically associated chiefly with the early cleavage products of the tuberculin protein and cannot be removed by washing, but is released at  $p_H$  4.8. Nearly one fourth of the polysaccharide in tuberculin is so associated. The rest of the polysaccharide, not bound to the protein, passes even the finest filter used in these experiments. With this information it has been possible to prepare in quantity an extremely potent tuberculin protein in the form of a powder, which is practically completely water-soluble

and therefore undenatured, and which has a nitrogen content of 16 per cent and a polysaccharide content of 2.7 per cent or less. Only 0.01 mg. is required to produce a maximum skin reaction in sensitive tuberculous guinea-pigs, and 5 mg. is sufficient to kill such a guinea-pig in twenty-four hours. It gives a very high precipitin titer with serums from rabbits immunized with the protein-carbohydrate complex of tuberculin (that is, the whole ammonium sulphate precipitate of tuberculin prepared from the human type of tubercle bacillus). The primary toxicity for normal guinea-pigs is extremely low—about that of timothy bacillus protein.

H. J. CORPER.

EXPERIMENTAL TUBERCULIN PNEUMONIA. ALFRED LARSON and ESMOND R. LONG, *Am. Rev. Tuberc.* **23**:41, 1931.

The authors used a purified tuberculin prepared by ultrafiltration from a human culture on Long's synthetic medium and tested this by intratracheal injection into tuberculous and nontuberculous guinea-pigs. The sharp differentiation expected in the effect of tuberculin protein on the lungs of normal and tuberculous animals was not realized. In the alveoli of both groups a cellular exudate was produced. A quantitative difference in the normal and sensitized animals was found, however: the exudate caused by the tuberculin tended to clear up in from forty-eight to seventy-two hours, leaving the lung normal, and in no instance was caseous necrosis noted.

H. J. CORPER.

MONOCYTES IN TUBERCULOUS ANIMALS. J. T. GEIGER, *Am. Rev. Tuberc.* **23**:76, 1931.

In the normal nontuberculous animal there is no reservoir of monocytes capable of supplying great numbers of these cells on acute demand. Such a reservoir can be and is established in tuberculous animals. After its establishment the monocytes respond to acute stimuli in a manner similar to that of the granulocytic leukocytes.

H. J. CORPER.

DO. BROTH CULTURE FILTRATES CONTAIN A BACTERIAL GROWTH-INHIBITING SUBSTANCE? L. A. BARNES, *J. Bact.* **21**:395, 1931.

The results of the experiments described do not support the observations of Besredka that filtrates of broth cultures contain a substance, liberated from the microbial cell, that inhibits bacterial growth. When filtrates prepared from broth cultures, pancreatin-digested broth or bacterial cell solutions are added in varying proportions to nutrient broth, the resulting diminution in the growth of the inoculated organisms is comparable to, or even less than, that observed when distilled water (or saline solution) is used as the diluent. It appears to the writer, therefore, that the effect of culture filtrates on the growth of bacteria is due to an alteration of necessary nutrient materials rather than to the presence of an inhibitory agent.

AUTHOR'S SUMMARY.

THE ETIOLOGY OF INFECTIOUS DIARRHEA (WINTER SCOURS) IN CATTLE. F. S. JONES and R. B. LITTLE, *J. Exper. Med.* **53**:835, 1931.

A disease of cows manifested by severe diarrhea is described. The condition is characterized by the frequent passage of dark brown or black feces, often containing mucus and blood. The principal lesions are catarrhal inflammation of the small intestine and degeneration of the liver. By feeding feces from cows spontaneously infected to calves a similar, but milder, disease, characterized by the same type of enteritis, was produced. Vibrios were cultivated from the inflamed intestinal tract in such experimentally induced cases. Pure cultures of the vibrios when fed to other calves, in certain instances, produced diarrhea and a well marked



enteritis similar to that observed both in the spontaneous disease and in calves following the feeding of feces from naturally infected cows. Vibrios were recovered from the inflamed small intestine of three of four animals fed such cultures.

AUTHORS' SUMMARY.

VIBRIONIC ENTERITIS IN CALVES. F. S. JONES and R. B. LITTLE, J. Exper. Med. **53**:845, 1931.

An intestinal disorder of calves is described. The clinical manifestations are usually observed in calves 2 or more weeks old. Our experiments indicate that infection may take place relatively early in life and may for a time produce only a mild reaction, but as the disease becomes more chronic, the clinical manifestations become more pronounced. The anterior portion of the jejunum is the primary locus of infection, but in more chronic cases practically the whole small intestine may be involved. Vibrios were cultivated from the inflamed intestinal mucosa in both the acute and the more chronic spontaneous cases. Vibrios were also obtained from the acutely involved intestines of young calves experimentally exposed to natural infection. On three occasions similar vibrios were found in cultures from the liver. When a single strain of vibrios that had been under cultivation for three months was fed to a young calf, subclinical infection was produced, and the organism was recovered. This strain after three passages on culture medium, when fed to a calf, produced a severe inflammation of the jejunum and ileum, and from these areas the organism was recovered.

AUTHORS' SUMMARY.

VIBRIOS (*VIBRIO JEJUNI*, N.SP.) ASSOCIATED WITH INTESTINAL DISORDERS OF COWS AND CALVES. F. S. JONES, M. ORCUTT and R. B. LITTLE, J. Exper. Med. **53**:853, 1931.

A number of vibrios obtained from the small intestines of calves fed feces from cows with spontaneous diarrhea, the natural intestinal disorders of calves, the experimentally induced infections of calves and cultures obtained from Dr. Theobald Smith were studied. From the close morphologic resemblance of the cultures, the similarities in motility, position and number of flagella, the tinctorial properties, and the tendency to fragmentation in older cultures, as well as the narrow nutritive requirements, we are led to regard them as a closely allied group, and we propose the name *Vibrio jejuni*. Immunologically, as judged by agglutination, the organisms have been divided into two groups, the smaller representing two strains originating from diarrhea in cows and the larger comprising one from this source and many from the disease in calves. The larger group can be subdivided by means of agglutinin absorption into cultures that do not contain the complete antigenic complex and others that do. Certain freshly isolated vibrios, when injected into rabbits, incite definite reactions terminating in a localization of the vibrios in the small intestine accompanied by catarrhal inflammation.

AUTHORS' SUMMARY.

BARTONELLA MURIS ANEMIA. D. PERLA and J. M. GOTTESMAN, J. Exper. Med. **53**:869 and 877, 1931.

The pathologic changes that follow a severe infection of the adult or young albino rat with *Bartonella muris* are of three kinds. First are those that result from the release of large quantities of cellular debris into the circulating blood. These include phagocytic activity and hyperplasia of the endothelial elements of the liver, thymus, lymph nodes and, in the young rat, of these elements in the spleen, with resultant capillary thromboses and focal necroses. Second, changes result from the anemia as such—fatty metamorphosis of the heart, liver and kidneys. Third, there is a severe nephrosis within some instances, a degenerative process in the glomeruli. In the bone marrow, hyperplasia of erythropoietic elements occurs.

In carriers of *Bartonella muris* certain changes are observed in the lymphoblastic, reticular and endothelial elements after splenectomy and recovery from the anemia. These changes appear from three to five months after splenectomy and are associated with immunity to further infection with *Bartonella muris*. The changes consist primarily of hyperplasia of hemolymph tissue, hyperplasia of the reticular and endothelial elements of lymph nodes, the formation of lymphoblastic foci periportally in the liver and peribronchially and perivascularly in the lung, regeneration of all elements of the thymus and marked hyperplasia of all elements of the bone marrow (increased hematopoiesis).

AUTHORS' SUMMARY.

THE ACCUMULATION OF IRON IN TUBERCULOUS AREAS. V. MENKIN and M. F. MENKIN, J. Exper. Med. **53**:919, 1931.

Daily intravenous injections of ferric chloride solution are followed by an accumulation of iron in tuberculous areas of the lungs. The iron accumulates in the caseous portions of the tubercles and is demonstrable by the prussian blue reaction. Quantitative determinations corroborate these results, and show that the iron content of lung tissue in tuberculous animals given injections of ferric chloride exceeds that in normal animals given injections of this salt, as well as that in tuberculous animals not given the injections.

AUTHORS' SUMMARY.

TICKS AS VECTORS OF TYPHUS FEVER. H. ZINSSER and M. R. CASTANEDA, J. Exper. Med. **54**:11, 1931.

Mexican typhus virus can be passed through ticks by the method of rectal injection. The virus remains alive in the ticks for at least twelve days. These studies, with one of our preceding publications and the work of Dyer, demonstrate that there are at least three insects—bedbugs, fleas and ticks—which must be considered as possibilities in the conveying of typhus fever from an animal reservoir to man. Our work will be continued by a study of rats and mice caught in typhus regions, such as Mexico City and its immediate vicinity, with a search for the virus in these rodents, as well as an analysis of the insects found on them or in the localities in which they are concentrated.

AUTHORS' SUMMARY.

TRANSMISSION AND CULTIVATION EXPERIMENTS WITH TRACHOMA. P. K. OLITSKY, R. E. KNUITTI and J. R. TYLER, J. Exper. Med. **54**:31, 1931.

Conjunctival tissue derived from alien and native American white persons having trachoma in an advanced stage has been used successfully to induce in monkeys of the species *Macacus rhesus* characteristic granular conjunctivitis. The transfer of infection was effected either by a single subconjunctival injection or by repeated swabbings with conjunctival secretions. Pathogenic strains of *Bacterium granulosis* have been recovered from the trachomatous tissues of six of eleven patients. In addition, the organisms have been isolated from the monkeys infected with the human material. Repeated swabbings with secretions obtained from monkeys having experimental trachoma have given rise to characteristic granular conjunctivitis in normal animals. In addition, repeated instillations of suspensions of fragments of conjunctival tissue derived from affected monkeys have led to a characteristic infection of the conjunctivae of normal monkeys. Contact infection occurs in monkeys, as it has long been known to occur in human beings, animals with smooth conjunctivae acquiring the experimental disease when merely caged with infected monkeys. Repeated instillations of cultures followed by rubbing of the eyelids lead to the disease in monkeys, a method of transfer that indicates one manner in which the disease may be transmitted from man to man. Yet another manner of producing the experimental condition is by repeated swabbings with cultures of *B. granulosis*. Noguchi has reported the successful outcome of the subconjunctival inoculation of cultures and the spread of the disease from an infected

conjunctiva to the other eye of the same animal. Tissues derived from human beings with trachoma or from monkeys having the experimental disease induce, on conjunctival inoculation into monkeys of the species *Macacus rhesus*, the same clinical and pathologic effects as do cultures of *B. granulosis*. The conjunctival lesions closely resemble, in clinical appearance and in microscopic changes, those of the follicular stages of trachoma in man.

AUTHORS' SUMMARY.

EXPERIMENTAL DERMAL PNEUMOCOCCUS INFECTION IN THE RABBIT. C. P. RHOADS and K. GOODNER, *J. Exper. Med.* **54**:41, 1931.

Experimental dermal pneumococcal infection in the rabbit is described in detail, and the histologic alterations are compared with those seen in the pneumonic lung in man. There would appear to be a basic similarity of the lesions in both tissues. A copious production of edema fluid is the outstanding characteristic of the early lesion. It occurs prior to any significant cellular change. In the spreading lesion an infiltration of the tissues with fluid precedes any other sign of reaction between tissue and microorganisms. It seems likely that the advancing fluid carries with it the infecting organisms and inoculates all tissues that it reaches. The resulting infection seems not to take place by an active invasion of micro-organisms, but by a progressive inoculation from an infected fluid.

AUTHORS' SUMMARY.

THE ACTION OF A BACTERIAL ENZYME ON TYPE III PNEUMOCOCCUS. O. T. AVERY and R. DUBOS, *J. Exper. Med.* **54**:51 and 73, 1931.

An organism has been isolated from peat soil which decomposes the specific capsular polysaccharide of pneumococcus type III. The isolation has been made possible by the use of a synthetic mineral medium containing the specific polysaccharide as the sole source of carbon. By repeated transfers in this medium the capacity of the organism to decompose the specific substance has been progressively increased. The organism is a pleomorphic bacillus, motile and spore-bearing, exhibiting metachromatic granules; its reaction to Gram's stain varies according to the medium on which it is grown. It is strictly aerobic, and grows well in plain broth and peptone solutions; it does not produce gas in any medium, and it forms small amounts of acid only on dextrine, galactose, lactose, salicin and trehalose; its growth is inhibited by dextrose. The organism decomposes the capsular polysaccharide of pneumococcus type III aerobically, between  $p_H$  6.2 and 7.8, at room temperature and at 37.5 C., but not at 54 C. The decomposition of the specific substance is inhibited by the presence in the medium of other nutrients, such as peptones, which act as a more readily available source of energy. The action of the organism is specific; it does not attack the soluble specific substance of type I or type II, nor any of the other bacterial polysaccharides thus far tested. The organism possesses an endocellular enzyme. This enzyme has been extracted by autolysis of the bacterial cells; in sterile solution it exhibits the same specific action as do the organisms from which it is derived, decomposing only the capsular polysaccharide of pneumococcus type III. This enzyme decomposes the specific polysaccharide of type III under anaerobic, as well as under aerobic, conditions; it is inactivated at from 60 to 65 C.; the rate of decomposition of the specific substance is not affected by the presence of normal serum. There exists a quantitative relationship between the total amount of specific substance decomposed and the amount of enzyme preparation used; the existence of this relation makes it possible to express the activity of a given enzyme preparation in terms of the minimal amount required for the complete decomposition of a given amount of specific substance. The specific decomposition of the capsular polysaccharide of pneumococcus type III, by the organism as well as by the enzyme it produces, illustrates once more the specificity of the types of pneumococcus and confirms the fact that the capsular polysaccharides, and not some impurities carried along with them, are responsible for type specificity.

The bacterial enzyme that decomposes the purified capsular polysaccharide of pneumococcus of type III in vitro also destroys the capsules of the living organisms growing in culture mediums and in the animal body. Potent preparations of this same enzyme protect mice against infection with virulent type III pneumococcus. The protective action is type-specific. The protective activity of the specific enzyme is destroyed by heat (70 C. for ten minutes). The enzyme remains in an effective concentration for from twenty-four to forty-eight hours after its injection into normal mice. The enzyme has been found to exert a favorable influence on the outcome of an infection already established at the time of treatment. A definite relationship has been found to exist between the activity of the enzyme in vitro and its protective power in the animal body. The mechanism of the protective action is discussed with special reference to the relation between the decapsulation of the bacteria by the enzyme and the phagocytic response of the host.

## AUTHORS' SUMMARIES.

PSITTACOSIS. T. M. RIVERS, G. P. BERRY and D. H. SPRUNT, J. Exper. Med. 54:91, 105, 119 and 129, 1931.

*Experimental Infection in Parrots (Rivers, Berry and Sprunt).*—The virus of psittacosis is present in the nasal secretions, feces, blood, spleens and livers of infected parrots. Parrots are susceptible to intra-oral, intranasal or intramuscular inoculations of the virus. The most constant pathologic changes produced by psittacosis in parrots occur in the spleen and the liver. The lesions in the latter organ consist of areas of necrotic liver cells and damage to bile ducts. In no instance, in our experience, were lesions observed in a parrot's lungs comparable to those found in the lungs of man. "Minute bodies" similar to those described by Levinthal and others were found in many, but not in all, of the infected birds. Parrots that have recovered from one attack of psittacosis exhibit an active immunity against reinfection.

*Experimental Infection in Mice (Rivers and Berry).*—The work presented in this communication concerning psittacosis in mice confirms Krumwiede's observations that mice inoculated intraperitoneally with emulsified livers and spleens containing the virus acquire the disease, and that the malady can in this way be passed serially through a number of mice. Furthermore, it has been shown that mice are susceptible to the virus administered intracerebrally, and that the active agent can be propagated indefinitely by means of brain to brain inoculations. Moreover, by the use of mice, the presence of the virus of psittacosis in the sputum of a patient with the disease has for the first time been demonstrated. It follows that the mouse is available for diagnostic purposes. The pathologic findings in infected mice consist of enlarged fatty livers that frequently show areas of necrosis infiltrated with polymorphonuclear and mononuclear cells; enlarged spleens with areas of necrosis and cellular infiltrations involving the pulp and lymphoid follicles, and, finally, intracerebrally infected animals, a meningo-encephalitis. The "minute bodies" described by other observers were not found in all animals, but they were seen with sufficient frequency in smears of peritoneal and meningeal exudates and in smears and sections of livers and spleens to demand serious consideration as the possible etiologic agent of the disease. Neutralizing and protective antibodies were not found in convalescent human serums when the mouse was used as the test animal.

*Experimental Infection in Rabbits and Guinea-Pigs (Rivers and Berry).*—Rabbits and guinea-pigs are susceptible to psittacosis virus introduced intracerebrally. By means of brain to brain passages in these animals the active agent is capable of propagation indefinitely. Serial passages of the virus through rabbits and guinea-pigs do not cause the active agent to lose its pathogenicity for parrots and mice. The chief clinical evidences of infection in rabbits and guinea-pigs following intracranial inoculation of the virus are fever and loss of weight. The pathologic changes are characterized by a mild meningo-encephalitis, and fatty

degeneration, focal necrosis and infarction of the liver. Rabbits on recovery from an attack of psittacosis are actively immune. Two strains of virus, human and parrot, were found to be immunologically similar. No evidence was obtained to show that human convalescent serum possesses an appreciable amount of neutralizing substances.

*Experimental Infection in Monkeys (Rivers and Berry).*—The virus of psittacosis inoculated intratracheally or intranasally in monkeys produces a pneumonia similar to that caused by the same active agent in man. Intracerebral inoculation of the virus induces a meningo-encephalitis characterized principally by a mononuclear reaction in the meninges. Indirect evidence has been adduced to show that the portal of entry of the virus in man is the upper respiratory tract.

#### AUTHORS' SUMMARIES.

SPORES OF *CLOSTRIDIUM TETANI*. T. J. MURRAY and M. R. HEADLEE, *J. Infect. Dis.* **48**:436, 1931.

Effective temperatures for the destruction of spores of *Clostridium tetani* in physiologic solution of sodium chloride varied from 105 to 95 C. The time varied from five to sixty minutes, three strains resisting heating for sixty minutes at 95 C. Effective temperatures for dry spores varied from 140 to 125 C., and the time from five to forty minutes. Dried spores heated under moist conditions showed no increase in resistance. Spores in buffer solutions showed the best resistance at  $p_H$  7. Spores in 1 per cent peptone solution were also more resistant at  $p_H$  7. Increasing amounts of organic matter (peptone) tended to increase the thermal death time at  $p_H$  7. Spores suspended in 2 per cent sodium chloride showed the greatest resistance to heat.

FROM AUTHOR'S SUMMARY.

SPORES OF *BACILLUS ANTHRACIS*. T. J. MURRAY, *J. Infect. Dis.* **48**:457, 1931.

Effective temperatures for the destruction of spores of *Bacillus anthracis* in physiologic solution of sodium chloride varied from 105 to 90 C. The time varied from five to forty-five minutes. Higher temperatures were necessary when dried spores were heated in the absence of moisture. Spores heated in buffer solution showed the best resistance at  $p_H$  8. In 1 per cent peptone solutions the resistance increased as the  $p_H$  increased. The resistance of spores suspended in sodium chloride solutions decreased with the increase in the concentration of the salt.

FROM AUTHOR'S SUMMARY.

SPORES OF *CLOSTRIDIUM WELCHII*. M. R. HEADLEE, *J. Infect. Dis.* **48**:468, 1931.

The resistance of spores of *Clostridium welchii* in physiologic solution of sodium chloride varied from thirty minutes at 90 C., to ten minutes at 95 C., and five minutes or less at 100 C. Spores were more tolerant of acid than of alkali. Optimum resistance of spores was found in 3 per cent sodium chloride solutions. Spores in peptone and gelatin showed resistance inversely proportional to the concentration of organic substance. Optimum resistance was shown in 1 per cent starch solution. Icebox temperature had little effect on the thermal resistance of the spores. Dry spores were found more resistant to heat than wet spores.

FROM AUTHOR'S SUMMARY.

INFECTION WITH *BRUCELLA ABORTUS*. A. THOMSEN, *J. Infect. Dis.* **48**:484, 1931.

Two hundred and seventy-two persons in contact with cattle were examined for agglutinins and complement-fixing antibodies for *Brucella abortus*. Individuals in contact with sick animals showed a large percentage of positive reactions, but a history of undulant fever was noted in only one case. Complement-fixation tests gave more positive results than the agglutination test. Among the persons handling

flesh of killed animals and milk, few positive reactions were found. A control group of sixty-one healthy patients and nonfebrile patients showed no positive reactions.

EDNA DELVES.

OTITIS MEDIA AND MASTOIDITIS DUE TO STREPTOCOCCUS EPIDEMICUS.

I. PILOT, M. LAMPERT and D. J. DAVIS, *J. Infect. Dis.* **48**:498, 1931.

Six cases of otitis media and four of mastoiditis due to *Streptococcus epidemicus* are described. The complications due to this streptococcus may follow sporadic sore throat, scarlet fever or mild infections of the upper respiratory tract.

AUTHORS' SUMMARY.

TONSILLECTOMY IN CARRIERS OF STREPTOCOCCUS EPIDEMICUS. I. PILOT and D. J. DAVIS, *J. Infect. Dis.* **48**:501, 1931.

Tonsillectomy in carriers of *Streptococcus epidemicus* causes rapid disappearance of this organism from the throat. Persons presenting sporadic cases of septic sore throat, otitis media and mastoiditis often become carriers of *S. epidemicus* in tonsillar crypts. *S. epidemicus* may cause acute infection in tonsillectomized persons, but the organisms disappear during convalescence. *S. epidemicus* was found in the crypts of 13.8 per cent of extirpated tonsils. All of the patients gave a history of sore throat. Many whose tonsils revealed *S. epidemicus* had cervical adenitis, arthritis or arthritic pains. The removal of the tonsils in these patients resulted in marked clinical improvement.

AUTHORS' SUMMARY.

SPORADIC CELLULITIS AND ABSCESS DUE TO STREPTOCOCCUS EPIDEMICUS.

I. PILOT and D. J. DAVIS, *J. Infect. Dis.* **48**:505, 1931.

Cellulitis and deep subcutaneous abscess may be due to *Streptococcus epidemicus*. Abscesses in the cervical lymph glands may follow sore throat due to this organism. Peritonsillar abscess, suppurative otitis media, mastoiditis, meningitis and empyema may also be caused by *S. epidemicus*. These infections appear to be sporadic and not associated with milk-borne epidemics of sore throat.

AUTHORS' SUMMARY.

DISSOCIATION OF HEMOLYTIC STREPTOCOCCI. R. TUNNICLIFF, *J. Infect. Dis.* **48**:511, 1931.

Many strains of hemolytic streptococci from erysipelas, scarlet fever and septic sore throat remain stable for years, both immunologically and culturally. A few strains dissociate, and the cocci from the dissociated colonies differ from those in the original culture in colony formation, immunity reaction, sometimes in production of color on chocolate agar, and in virulence. Many of the dissociated cultures may revert to the original type of streptococcus.

AUTHOR'S SUMMARY.

EPIDEMIOLOGY OF UNDULANT FEVER. C. F. JORDAN, *J. Infect. Dis.* **48**:526, 1931.

Agglutinins for *Brucella melitensis* in the group of the general population studied appear to be due to the use of raw dairy products. The group of veterinarians show a higher percentage of positive agglutinin reactions, owing to their direct contact with cattle. Infection with *Br. melitensis* is in direct proportion to exposure. Infection due to the ingestion of raw dairy products seems dependent on the amount consumed, the duration of exposure and the number of organisms ingested. The number of "no agglutination" reactions in persons following infection associated with disease and infection without disease indicate the probable rôle of immunity in the epidemiology of undulant fever.

EDNA DELVES.

FECAL FLORA OF ADULTS. A. G. SANBORN, J. Infect. Dis. 48:541, 1931.

The fecal flora was characteristic of the individual. This was particularly true of the *Bacillus coli*-*Lactobacillus acidophilus* ratio. The majority had *L. acidophilus* in the feces. Sporulating anaerobes, a marked evidence of proteolytic activity, usually accompanied a high count of *L. acidophilus*. Presumably non-sporulating anaerobes were found in considerable numbers. The frequent presence of a small, gram-negative, anaerobic bacillus which seemed to affect the ratio of gram-negative to gram-positive bacilli is mentioned. High and low dilution plates, incubated under increased carbon dioxide tension, were used. EDNA DELVES.

BRUCELLA ABORTUS INFECTION. K. T. SASANO, D. CALDWELL and E. M. MEDLAR, J. Infect. Dis. 48:576, 1931.

Serums of 1,000 persons revealed positive complement-fixation for *Brucella abortus* in 96 and positive agglutination in 78. In only five cases was the diagnosis of undulant fever made. In all these cases, complement-fixation was positive, and the titers of agglutination ran from 1:135 to 1:200. In rabbits the complement-fixing substances and the agglutinins developed at about the same time, but the former persisted longer. Complement-fixation and agglutination remain for years after apparent recovery from the infection. A diagnosis of undulant fever based on positive complement-fixation and low titers of agglutination in the absence of positive blood cultures, is doubtful. EDNA DELVES.

BACILLUS HEMOLYTICUS. L. R. VAWTER and E. RECORDS, J. Infect. Dis. 48:581, 1931.

Agglutinins for *Bacillus hemolyticus* were specific for the species. One of the sixteen strains studied did not belong to the same agglutination group. Cross-agglutination of *B. hemolyticus* serums did not occur with antigens of *B. chauvaei*, *B. welchii*, *Vibrio septique* (types I, II and III) and three strains of *B. sordelli*. Agglutinating serums of other pathogenic anaerobes did not agglutinate *B. hemolyticus*. Antigens of *B. novyi* were unstable, but *B. novyi* serum failed to protect animals inoculated with *B. hemolyticus*. EDNA DELVES.

DIPHTHERIA TOXIN. M. E. MAVER, J. Infect. Dis. 49:1, 1931.

Chemical tests indicate that a protein is synthesized simultaneously with the production of toxin by diphtheria bacilli cultured in a synthetic medium. Experimental evidence that the toxic fraction is identical with or associated with a protein is as follows: In an electric field both toxin and protein migrate to the anode at  $p_H$  4.05 and to the cathode at  $p_H$  3.75. The protein and the toxin are simultaneously destroyed by trypsin and pepsin. Less action in destroying the toxin on the part of neutral trypsin, as compared with alkaline trypsin, might be taken as evidence that the toxic fraction is a whole protein. The production of crystalline protein toxin will be conclusive evidence.

FROM AUTHOR'S SUMMARY.

FECAL FLORA OF ADULTS. A. G. SANBORN, J. Infect. Dis. 49:37, 1931.

The degree of transformation of the fecal flora in an acidophil direction by means of a specific carbohydrate diet is dependent on the original presence of appreciable numbers of *L. acidophilus* and the stability of the flora already present. A diet high in carbohydrate and one high in meat protein tend to simplify the aerobic fecal flora, the one in the acidophil, the other in the colonic direction. Non-lactose-fermenting gram-negative bacilli developed in persons with a strongly colonic flora resistant to transformation. Proteolytic activity may be reduced by decreasing the meat protein and by increasing the lactose in the diet, even when

there is only a slight increase in *L. acidophilus* in the feces. *L. acidophilus* in the feces is more easily increased in persons under 40 years of age. Persons irregular in their defecations often eliminate large quantities of *L. acidophilus*. Foreign strains ingested in *L. acidophilus* milk completely suppressed native strains. After milk therapy was stopped, the foreign strains soon disappeared, with a return of the native *L. acidophilus*. *L. bulgaricus* was not isolated from the feces of three persons, but in one case *L. bulgaricus* milk appeared to decrease the number of native *L. acidophilus*. It is suggested that in adults *L. acidophilus* milk may be more valuable therapeutically than an attempted transformation of the fecal flora by appropriate diets.

EDNA DELVES.

BACTERIUM GRANULOSIS IN RELATION TO TRACHOMA: ITS PATHOGENICITY FOR VARIOUS MONKEYS AND APES. CHARLES WEISS, Tr. Am. Acad. Ophth., 1930, p. 233.

Working with three strains of *Bacterium granulosis* provided by the Rockefeller Institute, and later with two others isolated by ourselves, we have been able to produce only a transient type of follicular conjunctivitis, lasting not more than four months, in various types of monkeys and apes. We employed twenty-eight rhesus monkeys, two of the genus *Macacus inuus* (Algerian magots), one baboon, one callitriche and two chimpanzees. Of the twenty-eight rhesus monkeys, eight developed granular conjunctivitis. The two Algerian magots developed similar follicles. One baboon was inoculated three times, and transient follicles were induced twice, while the callitriche, which was inoculated once, remained free from infection.

In two young chimpanzees inoculated with the Rockefeller cultures of *Bacterium granulosis* only transient follicles were induced, whereas in a third young chimpanzee infected with fresh "virus" obtained from human trachomatous eyes advanced lesions developed, which contained Prowazek-Halberstaedter bodies, and both clinically and histologically showed a striking resemblance to the lesions of active human trachoma. Thus, eighteen days after infection, the mucous membranes of the lower conjunctiva were thrown into folds; there were papillary hyperplasia and hypertrophy, secretion and ptosis of the upper lid. The infection spread spontaneously to the uninoculated eye, and follicles developed in the upper and lower conjunctivae of both eyes, including the tarsi and retrotarsal folds. Attempts to isolate *B. granulosis* from the material removed from the conjunctival lesions of the patient, as well as from those of the chimpanzee, were unsuccessful. Histologic sections of the conjunctivae of the inoculated eye of this chimpanzee, taken on the two hundred sixteenth day after injection (when the animal died of generalized tuberculosis), showed roughening of the conjunctival surfaces, epithelial invaginations, lymphocytic infiltration, young fibroblasts and new connective tissue containing many small capillaries. Near the upper end of the tarsus, the layer of infiltrated inflammatory cells was thicker and contained plasma cells and histiocytes. The lids of the uninoculated eye showed a similar picture. The centers of the follicles contained groups of epithelioid cells.

AUTHOR'S SUMMARY.

FILTRABILITY AND POLYMORPHISM OF TUBERCLE BACILLI. A. FONTES, Beitr. z. Klin. d. Tuberk. 77:2, 1931.

Intracellular chromatin granules of tubercle bacilli can propagate without the rest of the bacterial body. The granular form is a phase in the life cycle of the organism. The life cycle is represented essentially by the following phases: granular dust, free granules, multiplication of granules, cellular organization, production of intracellular granules, production of extracellular granules, propagation of bacterial cells. The granule is the living entity that can produce infection. Tuberculous virus may be present in the animal body without producing the



characteristic tissue reactions. The virulence is variable, as shown by its decrease after filtration. Scrofulosis and hereditary infections are due to a special phase of the virus.

MAX PINNER.

FILTRABLE FORMS OF TUBERCLE BACILLI. A. VAUDREMER, Beitr. z. Klin. d. Tuberk. **77**:16, 1931.

The tubercle bacillus is not always acid-fast. It possesses a true endotoxin. Tuberculin is an exogenous poison and can produce sensibilization. The tubercle bacillus is very variable. When it is grown at low temperature and with a minimum of nutrient material, atypical forms are developed. In the life cycle of the organism there occurs a stage at which the nuclear substance seems to be in an amorphous state. Between this amorphous state and the visible granules there is a stage in which the virus is filtrable.

MAX PINNER.

VARIABILITY OF THE TUBERCLE BACILLUS. S. S. MAHER, Beitr. z. Klin. d. Tuberk. **77**:40, 1931.

Atypical strains of tubercle bacilli can be produced by culturing tubercle bacilli over long periods of time in liquid mediums. Out of the sedimented bacterial mass one obtains nonacid-fast granulated rods that frequently produce pigment. Similar variations can be produced by letting the culture medium dry. A strain of acid-fast rods is demonstrated, which was derived from the acid-fast spores of a culture of *Bacillus subtilis*. It is the author's belief that bovine tubercle bacilli are descendants of *B. subtilis*.

MAX PINNER.

VARIOUS FORMS OF TUBERCLE BACILLI. R. PLA Y ARMENGOL, Beitr. z. Klin. d. Tuberk. **77**:47, 1931.

The author shows photomicrographs of various forms of tubercle bacilli. These include cocci of various sizes, long granulated filaments and streptococcus-like forms.

MAX-PINNER.

VARIATIONS OF THE TUBERCLE BACILLUS IN FORM AND ACTION. H. MUCH, Beitr. z. Klin. d. Tuberk. **77**:60, 1931.

The tubercle bacillus exists in essentially two forms, in that described by Koch and in that described by Much. The latter form is very variable; it may consist of thick spheres, dustlike granules or invisible forms. In the latter stage it is filtrable and may produce atypical types of infection, but the classic form can always be regenerated. Tubercle bacilli can be lysed by injecting them into growing plants. In some plants tubercle bacilli are completely lysed, so that they cannot be demonstrated microscopically or by infections of animals. In other plants tubercle bacilli die without being lysed. In other plants tubercle bacilli are neither lysed nor killed. And in still other plants tubercle bacilli remain alive, but lose their virulence.

MAX PINNER.

MORPHOLOGIC VARIATION OF TUBERCLE BACILLI. O. KIRCHNER, Beitr. z. Klin. d. Tuberk. **77**:72, 1931.

By growing tubercle bacilli submerged in liquid mediums atypical forms are obtained. The latter are shown in a colored illustration.

MAX PINNER.

THE DEVELOPMENTAL CYCLE OF THE TUBERCLE BACILLI. H. MOLLGAARD, Beitr. z. Klin. d. Tuberk. **77**:83, 1931.

If bovine or human tubercle bacilli are seeded on mediums containing filtered bone-marrow extract or autoclaved yeast extract, nonacid-fast and partly granu-

lated rods appear first. They are first gram-negative, but the granules, frequently in diplococci arrangement, become gram-positive. On prolonged culturing on these mediums, long filaments and sometimes typical actinomycetes with gonidia occur. The same forms were recovered from animals after infection. The same coryne-like and actinomycotic forms were obtained from the bone-marrow of tuberculous calves, and the same forms were recovered from the blood of human beings with pulmonary tuberculosis in the acute stage. The diplococcic forms grow out to rods, which are either gram-negative or gram-positive or acid-fast. The gram-positive rods develop on Petroff's medium into typical acid-fast rods. It is important in repeating the studies to adjust the mediums to  $pH$  7.3 and to keep them in an atmosphere of 5 per cent carbon dioxide. The bone-marrow for the mediums must be taken from very young calves; the yeast extract must be prepared from dry yeast. The nonacid-fast forms occur usually between the fourteenth and twenty-first days. The culture medium remains clear and contains granular matter, which usually adheres to the wall and to the bottom of the tube. All nonacid-fast forms were nonmotile.

MAX PINNER.

### Immunology

INTRAVENOUS VACCINATION WITH HEMOLYTIC STREPTOCOCCI. M. G. WILSON and H. F. SWIFT, *Am. J. Dis. Child.* **42**:42, 1931.

The seasonal incidence of the recurrence of rheumatic activity among 172 children of the susceptible age group observed for a period of four years is presented. About half of these children received intravenous vaccination with hemolytic streptococcal vaccine. The remaining number were observed as a control group. The incidence of recurrence and manifestations of activity in both groups were comparable during the years 1927 and 1928, before vaccination was given. The yearly incidence of recurrence was less in the treated group than in the control group during the years 1929 and 1930, after treatment. Forty-five per cent of the treated children, as compared with 18 per cent of the controls, were free from recurrence for periods of from sixteen months to two years after treatment. The causal relation of intravenous vaccination with hemolytic streptococcal vaccine to the diminished incidence of recurrence observed is discussed.

AUTHORS' SUMMARY.

THE INFLUENCE OF DEHYDRATION ON ANAPHYLAXIS IN GUINEA PIGS. M. I. RUBIN and C. E. KELLETT, *Bull. Johns Hopkins Hosp.* **49**:170, 1931.

Dehydration is shown to protect a sensitized guinea-pig from anaphylactic shock. The experiments with histamine suggest that the protection thus afforded is in part, at any rate, due to diminished irritability of smooth muscle. We have pointed out that dehydration may explain the success of many of the other anti-anaphylactic procedures. The clinical application of the results of our experiments are encouraging.

AUTHORS' SUMMARY.

THE SKIN AS AN IMMUNOLOGICAL ORGAN. L. TUFT, *J. Immunol.* **21**:85, 1931.

During the course of some experiments in serum-sickness, it was observed that the skin seemed to possess an unusual capacity for sensitization to horse serum, this sensitization being accompanied by the production of anaphylactic antibodies, and these experiments suggesting the possibility that the skin may act as an important immunologic organ. Numerous clinical observations bear out the close relationship existing between the skin and processes of immunity in infectious and allergic diseases. These have been further substantiated by many experimental contributions reported in the past few years in the foreign literature, which appear to

indicate not only that the skin may have the power to form antibodies, but that such antibodies may be specific and that the ability to form such antibodies resides specifically in the skin. My own experiments do not bear out the latter view, at least for antibacterial antibodies, for from a comparison of the antibody response after the intradermal, subcutaneous, intramuscular and intravenous injection of mixed typhoid vaccine, both in the human being and in the lower animals, it would appear that though it is possible that the skin participates actively in antibody production, it does not carry out this function exclusively in respect to other tissues or organs. The marked antibody response particularly after local injection can be best explained on the basis of a local tissue stimulation of the cells of the reticulo-endothelial system, these cells being particularly abundant in the skin. That the active antibody response after intradermal injection may be due in part to a slower absorption of the antigen is entirely possible, although this process may in turn allow for local fixation of a greater amount of antigen and thus greater local stimulation of antibody.

AUTHOR'S SUMMARY.

DIPHTHERIA ANTITOXIN IN HUMAN SWEAT. J. M. NEILL, E. L. GASPARI, R. A. MOSLEY and J. Y. SUGG, *J. Immunol.* **21**:101, 1931.

This paper deals with diphtheria antitoxin in human sweat. Significant amounts were found in the sweat of three men possessing large amounts in their blood (two with "natural" immunity and one previously given an injection of toxoid); none was found in the sweat of people with only small amounts of antitoxin or none in their blood. The data are compared with previous data on saliva and urine on the basis of the following questions: the comparative frequency of persons with detectable antitoxin in the secretion, comparative ratios to serum antitoxin, daily output and significance of sweat as a channel of loss of immune substances.

AUTHORS' SUMMARY.

A QUANTITATIVE ASPECT OF THE HYPOTHETICAL INCORPORATION OF INJECTED ANTIGEN IN RESULTING ANTIBODY. S. B. HOOKER and W. C. BOYD, *J. Immunol.* **21**:113, 1931.

From quantitative data relative to the weight of injected antigen and to the potency of resulting antibody, it is conservatively estimated that one molecule of active antigenic substance gives rise to an amount of agglutinin capable of flocking 600 bacteria. This result is difficult to reconcile with the hypothesis that antibody is a conjugate of antigen and body globulin. The surface relationship (implicated in the mechanics of agglutination) between one globulin molecule and 600 bacteria is 1:25,000,000. Even with the assumption of multiple monospecific determinants in a single molecule of antigen this discrepancy is extreme. A theory of antibody formation involving catalysis would seem more promising.

AUTHORS' SUMMARY.

ON THE MECHANISM OF TOXIN-ANTITOXIN REACTIONS. J. FREUND, *J. Immunol.* **21**:127, 1931.

High dilutions of tannin in a neutral or slightly acid menstruum detoxify diphtheria and tetanus toxins. The detoxifying effect of tannin can be demonstrated on either highly diluted toxins or toxins absorbed on collodion particles. There is a characteristic prezone in the effect of tannin on toxin. Dilutions from 1:100 to 1:3,200 are not so effective as higher dilutions. (The same prezone characterizes the agglutination of red blood cells by tannin.) These experiments seem to be in harmony with the view that the various reactions demonstrated with a given antigen are all due to the same antibody and that the mechanism of all antigen-antibody reactions is the same.

AUTHOR'S SUMMARY.

AGGLUTININ PRODUCTION IN SUPRARENALECTOMIZED RATS. D. KHORAZO, J. Immunol. **21**:151, 1931.

In contrast to previous work, we have been unable to demonstrate that bilateral suprarenalectomy in rats alters agglutinin production against typhoid vaccine.

AUTHOR'S SUMMARY.

TOXINS OF STREPTOCOCCUS EPIDEMICUS. I. E. PILOT and I. DREYER, J. Infect. Dis. **49**:135, 1931.

*Streptococcus epidemicus* of epidemic septic sore throat produces a toxin, as determined by intradermal tests; 98 of 324 persons (30 per cent) giving positive reactions. Susceptible persons, as well as rabbits, may be immunized with the epidemicus toxin. Skin reactions to the toxins of *S. epidemicus* are widely divergent from the reactions to the toxin of *S. scarlatinae*. Of 208 persons, 46 gave positive reactions to scarlet fever toxin, 56 to the epidemicus toxin and only 10 to both toxins. An attack of scarlet fever resulted in a loss of the skin reaction to the scarlet fever toxin, but did not affect the skin reaction to the epidemicus toxin. Of 24 patients convalescent from scarlet fever who gave negative reactions to the Dick test, 8 (33 per cent) reacted to the epidemicus toxin. In persons giving positive reactions to both toxins, immunization with scarlet fever toxin resulted in the disappearance of the susceptibility to the scarlet fever toxin, but did not affect the susceptibility to the epidemicus toxin; immunization with the epidemicus toxin resulted in loss of susceptibility to this toxin, while the result of the Dick test remained positive. Antiserums produced by means of the epidemicus toxin appear to be specific. They neutralize the epidemicus toxin, but do not affect the scarlet fever toxin in persons giving positive reactions to skin tests with both toxins.

AUTHORS' SUMMARY.

THE SPECIFICITY OF AVIAN TUBERCULIN REACTIONS. V. B. DOLGOPOL, J. Infect. Dis. **49**:216, 1931.

A positive reaction to avian tuberculin, observed in many cases of pulmonary tuberculosis, does not indicate an active mixed infection with human and avian tubercle bacilli. (This conclusion is based on the result of the inoculation of chickens with the sputum of ten patients who showed positive reactions to avian tuberculin). A positive reaction to avian tuberculin in pulmonary tuberculosis does not indicate latent infection with the avian tubercle bacillus. (This conclusion is based on the result of the inoculation of chickens with autopsy material, including peribronchial lymph nodes, from three patients who had shown positive reactions with avian tuberculin). The large percentage of positive reactions with avian tuberculin in tuberculous patients, observed by Maggiore, by Stewart and Collins, by L'Esperance, and by myself, must be considered only as a large percentage of group reactions in patients suffering from tuberculosis caused by the mammalian types of tubercle bacilli. In the presence of positive cutaneous reactions with mammalian tuberculins a positive reaction with avian tuberculin cannot be taken as indicating an infection with the avian tubercle bacillus; but such an infection may be assumed to exist when the reaction to avian tuberculin is positive while the reaction to other tuberculins is negative or considerably weaker. Tuberculosis does not develop in chickens when they have been inoculated intravenously or intraperitoneally with large amounts of mammalian tubercle bacilli.

AUTHOR'S SUMMARY.

THE NATURE OF THE ANTIBODIES. PAUL VON GARA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:1, 1931.

Ninety rabbits divided into small groups were given injections of ox serum, *Eberthella typhi* or sheep red blood cells. A few were treated with only one

antigen, but most were also given injections of the other antigens, and their serums were then studied for agglutinins, precipitins, hemolysins, complement fixing and bactericidal properties. With the exception of the bactericidal properties, which decreased, all the other antibodies showed a parallel increase, depending on the number of injections, two of which were the minimum necessary to produce a response. Anamnestic reactions following a single repeated injection of a homologous or heterologous antigen were noticed even when the interval between the injections of the two antigens was considerable. Following multiple injections of the new antigen, such anamnestic reactions could not be seen. The response to reinjections after intervals was also greater than to first injections. Absorption with the homologous antigen removed the antibodies completely, while absorption with heterologous antigen, though marked, was not equally complete, though parallel for all antibodies. The author concludes that the so-called different antibodies are only different reactive forms of the condition of the serum resulting from the injection of the antigens.

I. DAVIDSOHN.

BACTERICIDAL SUBSTANCES IN TISSUE CULTURES. SHUJI KOMATSU, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:76, 1931.

The formation of bactericidal substances was observed in cultures of the spleen and to a lesser extent of the bone-marrow of rabbits given injections of *Eberthella typhi*. The introduction of the antigen into the culture of the tissue was not followed by the development of bactericidal properties. They appear in the tissues, particularly in the spleen, earlier than in the blood serum. The development of bactericidal substances in tissue cultures of nontreated animals could not be observed.

I. DAVIDSOHN.

THE OCCURRENCE OF THE SEROLOGIC FACTORS M AND N IN THE JAPANESE. S. SHIGENO, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:88, 1931.

In 329 blood specimens sent from Japan to Berlin both factors were found in numbers similar to those observed in Europeans and whites of North America. A decrease in the titer, particularly of factor N, was observed in the transported blood specimens.

I. DAVIDSOHN.

THE SPECIFIC (IMMUNOLOGIC) PROPERTIES OF PURIFIED TOXINS. S. SCHMIDT, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:101, 1931.

Diphtheria anatoxins from various sources were successfully purified by adsorption with aluminum hydroxide. A reversal of the original detoxifying process with a return of the toxicity was not observed as a result of purification and concentration. Occasional slight increases of toxicity were, in the author's opinion, due to a concentration of a small fraction which was not detoxified during the preparation of the anatoxin. Besides the procedure of Ramon, a test for toxicity is suggested, consisting in an intracutaneous injection of 0.2 cc. into a rabbit. Purified and concentrated toxins and anatoxins showed better antigenic properties than the crude products. Good results were obtained when the immunization was begun with anatoxin and continued with toxin.

I. DAVIDSOHN.

## Tumors

NEUROGENIC SARCOMA. FRED W. STEWART and MURRAY M. COPELAND, *Am. J. Cancer* **15**:1235, 1931.

After a detailed study of the material of the Memorial Hospital the authors conclude that neurogenic sarcoma is an exceedingly variable disease. A large percentage of sarcomas of the soft parts are of neurogenic origin and possess the same gross and microscopic features and run the same clinical course as

so-called Recklinghausen's disease. The fundamental cell of origin of the tumor is the Schwann cell. The lamellar sheath contributes to certain of these tumors. Although they may occur anywhere, they have sites of predilection and a tendency to involve certain nerves. After excision they recur, as a rule. However, these recurrences are no other than new tumors arising from nerves in the vicinity. Evidence that irradiation after excision prevents or delays recurrence is lacking. The article contains a comprehensive discussion on the pathology of the tumors, and the reports of sixty-four selected cases of neurosarcoma from the literature in the form of tables. The references are abundant.

B. M. FRIED.

OLIGODENDROGLIOMA. S. T. KWAN and BERNARD J. ALPERS, Arch. Neurol. & Psychiat. **26**:279, 1931.

Kwan and Alpers give a detailed report of four cases of a type of a glioma classified by Cushing and Bailey as oligodendroglioma. They confirm the view that the latter is a specific, cellular and slowly growing tumor, consisting of glia or naked nuclei. These tumors are scattered in immense amounts in the subcortical white matter along the axons and are, of course, also present in the gray matter, mainly as satellites of the ganglion cells. With specific stains they show processes and are preferably designated as oligodendroglia or oligogliocytes. It is rather noteworthy that tumors should be made up of fully differentiated elements, as the oligodendroglioma is. Among oligogliocytes forming the latter, Kwan and Alpers also found unipolar and bipolar spongioblasts and transition forms between oligogliocytes and astrocytes. In some instances they were able to show the development of both oligogliocytes and the transition forms from the spongioblasts. An interesting feature was the presence, in one case, of tumor masses in the subarachnoid space, for which the authors, however, fail to give an adequate explanation.

GEORGE B. HASSIN.

MULTIPLE NEUROFIBROMATOSIS. K. HOSOI, Arch. Surg. **22**:258, 1931.

The author had been able to follow a benign neurofibroma through to a spindle cell sarcoma. Microscopic section of the lesion revealed a transformation from the relatively acellular neurofibroma to a cellular spindle cell tumor. Hosoi gives an extensive review of the literature. A total of sixty-five cases of sarcomatous transformation of neurofibromatosis have been collected. Seventy-two per cent of the cases occurred between the ages of 30 and 50. Malignant transformation takes place in about 13 per cent of all cases of Recklinghausen's disease. The tumor grows rapidly and tends to recur. Metastases are late. The prognosis is poor.

N. ENZER.

ADENOID CYSTIC CARCINOMA WITH GENERALIZED METASTASES IN THREE CASES OF THE BASAL CELL TYPE. A. W. SPIES, Arch. Surg. **21**:365, 1930.

The author reviews at considerable length the adenomatous types of basal cell tumor in the skin and mucosa, emphasizing the fact that true basal cell tumors of the mucosa are not universally accepted. The general conception that basal cell tumors are benign will have to be modified to the extent that basal cell tumors showing an adenoid structure may be malignant, as illustrated by three cases reported here: Spies has been unable to find an authentic, proved case of basal cell epithelioma with metastases. Most of those which have been reported as basal cell tumors with metastases were probably basal cell tumors with some squamous cell features. The adenoid cystic carcinomas of the skin are chronic, localized lesions, generally occurring at a younger age than the true basal cell carcinoma. They are radio-sensensitive. Those on the mucosa are much more malignant. These histologic features of both the cutaneous and noncutaneous groups are similar. The characteristic feature is the cordlike arrangement of cells of the basal type thrown into papillary and pseudoglandular formations. Occasional

solid alveolar forms and discrete cyst forms are found. Mitoses are infrequent. The formation of the cysts is open to some interpretation. Some authors believe that they are due to degeneration of the stroma. Others believe that they are results of secretory and desquamative activity of the cells themselves. In general, the adenoid structures of these tumors are poorly formed, and the unit is difficult to detect. The stroma is scanty.

N. ENZER.

CARCINOID TUMORS OF SMALL INTESTINE. H. H. COOKE, Arch. Surg. **22**:568, 1931.

Eleven cases are reported, three of which were malignant. The primary lesion was usually a circumscribed nodule. Multiple nodules were present in three cases. One of them formed an annular tumor. Nine of these were in the ileum and two in the jejunum. Metastases occurred in 20 per cent of reported cases, despite the fact that the histology of the carcinoid tumors generally suggests a benign tumor.

N. ENZER.

SARCOMA OF THE ESOPHAGUS. H. J. DVORAK, Arch. Surg. **22**:794, 1931.

Sarcoma of the esophagus is more frequent than carcinoma of the esophagus in young persons, its most frequent site being in the lower third. It may be a polypoid or solid medullary spindle cell tumor invading the wall. The author reports a case of rhabdomyosarcoma. About thirty cases have been reported in the literature.

N. ENZER.

INJURY AND GLIOMA OF BRAIN. H. L. PARKER and J. W. KERNOHAN, J. A. M. A. **97**:535, 1931.

A series of 431 cases of glioma of the brain have been studied in relation to injury to the head. Injury was reported to have occurred in 58 cases (13.4 per cent). As a control, in an equal number of cases without tumor of the brain there was a history of injury to the head in 45 (10.4 per cent). Two hundred normal persons were also interrogated, and 71 (35.5 per cent) reported injury to the head. With or without adjustment in patients with tumor for relatively greater liability to fall and be injured, the incidence of injury in this group is not sufficiently high, as compared to the other series, to suggest an etiologic factor. The relative incidence of injury in cases of glioma of the brain must therefore be abandoned as an argument in favor of injury being a cause of glioma. Considering the relatively high incidence of injuries to the head in the general population, there is ample possibility that the occurrence of the injury and the tumor in the same case may be mere coincidence.

Certain criteria are suggested to apply to a given case of glioma of the brain before the reported injury can even be assumed as a cause, especially in medico-legal cases. The 58 cases in which there was a claim of injury to the head subsequent to death or operation were found, in the light of these criteria, to shrink to 21, wherein, for the purpose of argument, injury might be assumed as cause. This represents only 4.2 per cent of the total series of gliomas of the brain. Therefore, in 95.8 per cent of the cases, injury had had played a part in the formation of tumor and accordingly cannot be a significant factor in the general causation of glioma. Further, in the cases considered here, both gross and microscopic examination of tissues failed to show any association between the tumor and an old injury.

Since the Great War, at least 2,858 cases of injury to the head, civil and military, have been reported in current literature. The patients have been observed for periods of from two months to twelve years. In none of the 2,858 cases was there a record of glioma of the brain developing as a result of these injuries. Therefore, glioma of the brain as a sequela of injury to the head must

be excessively rare and comes within the possibility of coincidence. The cause of glioma of the brain is still unknown, and as yet it is unwise to affirm that injury has any direct causal association, for the preponderance of evidence is against this conclusion.

AUTHORS' SUMMARY.

PRIMARY MALIGNANT ADAMANTINOMA OF THE OVARY. M. N. ZAJEWLOSCHIN, Frankfurt. *Ztschr. f. Path.* **41**:100, 1931.

A primary malignant adamantinoma of the ovary in a girl 8 years old is reported. During the operation for removal, it was noted that both tubes and the uterus were absent. The tumor weighed 200 Gm., and measured 6 by 7.5 by 9 cm. Its surface was in part smooth and in part nodular. On section, the bulk of the tumor consisted of an irregular lobular mass, a small amount of interstitial tissue and some calcified areas. A few cysts were also noted. Histologically, the tumor revealed typical adamantinoma structures. The author believes that the tumor reported is a congenital teratoma in which the predominant portions are represented by the adamantinoma. It is possible that originally this tumor was a dermoid cyst with tooth structures, from which the adamantinoma developed.

OTTO SAPHIR.

COLLISION TUMORS. P. GOETTING, Frankfurt. *Ztschr. f. Path.* **41**:107, 1931.

A pyloric tumor was removed from a man 38 years old. The liver, which was examined during the operation, showed no evidence of metastasis. A roentgen examination five months after operation revealed metastases in the lung. On palpation a metastatic tumor was also recognized in the liver. When the patient died subsequently, no autopsy could be obtained. Histologic examination of the pyloric tumor revealed a typical cylindric cell carcinoma and a spindle cell sarcoma which infiltrated the carcinoma. The various explanations of the histogenesis of these tumors are discussed.

OTTO SAPHIR.

CANCER IN HAMBURG. W. SCHWANKE, *Ztschr. f. Krebsforsch.* **32**:259, 1930.

Owing to the fact that immigrants to Hamburg consist almost entirely of young persons, the cancer statistics of that city permit of unusually close coordination as regards age incidence. There has been a great increase in late years of persons of advanced age. In 1900, 41 per cent of the population was over 30 years of age; in 1929, 53 per cent. During this period the general population has increased 1.6 times, while the incidence of cancer has risen 2.8 times. Under the age of 30 there has been no increase of cancer. The matter of age, however, is not held by Schwanke as the sole factor concerned in this increase, which he ascribes in part to the efficiency of modern prophylactic measures against general harmful agencies except such as contribute to malignancy. The rise in the cancer rate occurred not uniformly, but as successively higher waves. Despite the fact that the proportion of females in the population has increased, the relative incidence of cancer cases for this sex has decreased from 58 per cent for the period from 1872 to 1899 to 55 per cent for the period from 1902 to 1929. The statistics showed nothing in the way of a definitely reciprocal relationship between cancer and tuberculosis. The distribution by organs was as follows: In males 79 per cent, and in females, 54 per cent, were located in the alimentary tract; some decrease in cancer of the female genital organs was more than balanced by an increase of cancer in other organs, especially the breast. Even so, cancer of the aggregate sex organs was less frequent in the female sex than alimentary cancer, a shift in distribution that appears to have been in evidence ever since 1872. In males, on the other hand, there has been a slight diminution in the incidence of alimentary cancer. It would appear that diminished occurrence of cancer in one group of organs is associated with an increase elsewhere in the body.

H. E. EGGERS.



ADAMANTINOMATOUS TUMOR OF THE TIBIA. C. S. RICHTER, *Ztschr. f. Krebsforsch.* **32**:273, 1930.

There is here reported a tibial tumor of a Javanese in which the microscopic appearances were those of adamantinoma. This is the second case of the sort to be reported. The author regards it as a peculiarly differentiated bone endo-thelioma.

H. E. EGGERS.

### Medicolegal Pathology

COLLODION AS AN INSTRUMENT OF SUICIDE. M. MOSKOW, *Ann. de méd. lég.* **11**:78, 1931.

The lungs of a suicide were found to contain hardened collodion ramified throughout the bronchial tree. That the liquid evidently acted not as a poison but as a mechanical agent producing suffocation was demonstrated in an experimental study in which collodion was poured into the mouths of animals. In the majority, choking was followed by aspiration of the liquid and death, and hardened collodion was found in the respiratory passages. The suicide was complicated by the attempt of the victim to strangle himself with a strap, apparently in order to relieve the pain caused by penetration of the collodion into the lungs.

E. M. BARTON.

STATUS THYMICOLYMPHATICUS IN LEGAL MEDICINE. W. G. DABROWSKI, *Ann. de méd. lég.* **11**:128, 1931.

Studies were made of thymic and lymphoid tissue from 920 bodies. When death occurred quickly, whether due to suicide, to other violence or to fulminating disease, the thymus gland and lymphatic tissue were well developed in most young undebilitated persons, and no great difference in the development of those tissues was found in subjects of the same age group. A persistent thymus or so-called status thymicolymphaticus, therefore, cannot be considered proof of a predisposition to sudden death. Indeed, in these observations, proof of status thymicolymphaticus as an innate constitutional characteristic was lacking. Overdeveloped thymic and lymphatic tissue was not found more frequently in suicides than in other bodies when death had occurred quickly.

E. M. BARTON.

TRAUMATIC APPENDICITIS. P. WIART, *Ann. de méd. lég.* **11**:381, 1931.

True traumatic appendicitis is extremely rare. The lesions must be shown to be inflammation secondary to mild trauma in a hitherto normal organ. If traumatization is severe, the condition anatomically becomes classified with other abdominal contusions, whether the appendix was previously normal or diseased. All clinical manifestations and sequelae of these conditions should be attributed to the accident. When symptoms of appendicitis first appear at a time considerably removed from the accident the responsibility of trauma, provided there was a previously normal appendix, can rarely be established. In industrial accidents involving chronically inflamed appendixes only the immediate consequences of the accident can be admitted, while in "common law" accidents, the responsibility must be divided pro rata between the previous chronic state and trauma that conceivably might leave a normal appendix uninjured.

E. M. BARTON.

BLOODY SPINAL FLUID FOLLOWING AN OSTEOPATHIC TREATMENT. M. TRÉUEL, *Ann. de méd. lég.* **11**:488, 1931.

Several weeks after a "painful and violent" osteopathic treatment a woman, 34 years old, with a family history of syphilis and a negative Wassermann reaction of the blood had a violent attack of mania. In xanthochromatic spinal fluid

under increased pressure there was blood, albumin, a positive Wassermann reaction and bizarre changes in the colloidal gold curve. Three weeks later the cerebrospinal fluid was essentially normal. The author believes that the bloody fluid was of purely traumatic origin, due to the osteopathic manipulations.

E. M. BARTON.

APOPLEXY OR SUICIDE? FRITZ ARNDTS, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17**:164, 1931.

The precarious economic conditions in Germany, which have now extended over a long period of time, have led to many fraudulent actions against the insurance companies. Persons whose financial status is close to ruin take out high life insurance policies with accident indemnity, in order to protect their families financially, and, shortly afterward, under inexplicable or mysterious circumstances, their sudden death is reported. A pertinent case of this kind is presented in a thorough critical analysis, and it is recommended that in every similar instance an autopsy by competent medicolegal pathologists should be performed in order to clear the situation, with particular view in mind of a possible poisoning with modern hypnotics.

E. L. MILOSLAVICH.

MECHANISM OF SUDDEN DEATH FOLLOWING ACUTE CIRCULATORY DISTURBANCES. ERICH BRACK, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17**:176, 1931.

In instances of death due to a massive, suddenly developing hemorrhage, there is a contraction not only of both ventricles of the heart but also of large arterial vessels, particularly of the aorta. Thus, the blood is forced into the peripheral parts of the body, into the body cavities (internal hemorrhage) or to the outside (external hemorrhage), rapidly causing death. The parenchymatous organs do not show any marked signs of anemia, such as are constantly observed in cases of slowly occurring bleeding in the gastro-intestinal canal or from the female genital tract. Minute injuries to the walls of the heart, especially to the ventricles, or to the large arteries, such as the aorta, carotids, etc., may not cause any alarming symptoms and may gradually heal up. The simultaneously traumatized perivascular sympathetic plexus contracts the injured vessel, giving it a chance to cover the area with thrombotic material. Persons suffering with hypertension usually show profuse uncontrollable hemorrhages due to lack of the normal vasoconstrictive regulation, and the internal organs present higher degrees of loss of blood, as in instances of ordinary acute hemorrhages. Organs, such as the kidney, liver or spleen, may show a normal amount of blood if a topographically distant artery is severed. An abundant loss of blood (up to 2 liters) is endured far more easily by females than by males. The sudden constriction of the arterial system, as observed in various traumatic incidents, displaces the blood volume into the veins, and all the internal organs present a deeply cyanotic appearance, contributing to the suddenness of death. A similar mechanism and conditions are met with in cases of asphyxia due to mechanical obstruction of the upper respiratory tract. The rapidly developing increase of blood volume (plethora), as in acute alcoholism, is evidenced at autopsy by a dilatation of the entire arterial tree, and the left ventricle of the heart is dilated while the right ventricle is rather contracted and the cavae collapsed. In certain cases of fatal hemorrhage due to injury, one finds an extremely pale skin and testicles, while the internal organs exhibit a marked congestion. This abnormal blood supply is undoubtedly the effect of vasomotor action. Changes in the left branch of the conducting system of the heart, which often are difficult to establish, cause sudden death from a severe attack of the Adams-Stokes' type. *Cor bovinum*, which results from a miliary myocardial fibrosis, leads to unsuspected death because of an excessive, rapidly occurring blood supply to the myocardium. The so-called aorta angusta,

as found in certain cases of acute lethal circulatory disturbance, is the result of a vasomotor contraction. In a certain group of cases, diffuse dilatations of peripheral arteries, as those at the base of the brain or in the kidneys and heart, constitute the only anatomically detectable evidence of acute circulatory distress. Microscopic thrombosis of various organs, particularly of the brain, as seen in various poisonings, certainly forms the anatomic basis of sudden death. Pronounced congestion of the suprarenals at their corticomedullary boundary hinders the function and obstructs the transportation of the secretions of these vital organs, leading to their acute insufficiency.

E. L. MILOSLAVICH.

#### MEDICOLEGAL IMPORTANCE OF CERTAIN NEW ELECTROPATHOLOGIC FINDINGS.

A. D. KAPLAN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17**:217, 1931.

In the course of an electrical current of high tension, between the entrance and exit, one may find typical cutaneous burns on the surface of the body, particularly if the skin was folded or the extremities bent, as in the groin or armpit, such burns may indicate the position of the body at the time of accident. The spindle-like appearance of the cells of the malpighian layer is not characteristic of an electric mark, as similar changes are observed in ordinary burns, as, for instance, in those produced by a cauter. But changes of the various tissue structures beneath the epidermis, such as a marked elongation of the epithelial elements (protoplasm and nuclei) of the sweat glands, elongation of the endothelial cells of the capillaries and tears of blood vessels with perivascular hematoma, in connection with changes of the malpighian layer, are the result only of an electrical burn. The development of irregularly (zigzag) formed, tearlike channels in the hyalinized connective tissue of the cutis is of particular significance, as such formations do not occur in ordinary burns.

E. L. MILOSLAVICH.

#### COMPLICATIONS FOLLOWING ENDOCARDIAC INJECTIONS. WALDEMAR WEIMANN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17**:244, 1931.

Injections of epinephrine into the heart may lead to some unexpected complications. There are instances in which the internal mammary artery was punctured and a severe hemorrhage followed, or in which the lung was pierced and a pneumothorax developed. In the case described, subepicardial branches of the left coronary artery were punctured by repeated insertions of the needle, with the subsequent development of a hemopericardium.

E. L. MILOSLAVICH.

# Society Transactions

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## PATHOLOGICAL SOCIETY OF PHILADELPHIA

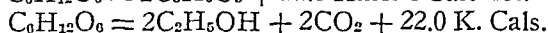
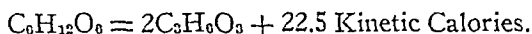
Oct. 8, 1931

BALDUIN LUCKÉ, *President, in the Chair*

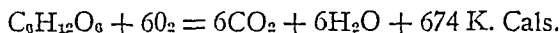
SOME PROBLEMS OF BACTERIAL RESPIRATION. MARJORY STEPHENSON,  
Biochemical Department, Cambridge University, England.

The fundamental problem which the anaerobic cell has to solve is that of finding an efficient substitute for oxidation by molecular oxygen as an energy-delivering process. Several mechanisms have been adopted for this purpose:

1. The exothermic fission of the hexose molecule into two three-carbon compounds. These subsequently rearrange themselves into stable compounds of lower energy content than the original hexose. The familiar alcoholic and lactic fermentations are examples of such anaerobic mechanisms for delivering energy:



Both are greatly inferior quantitatively to the oxidative mechanism:



2. Other mechanisms consisting essentially in replacing oxygen by some other substance to act as a hydrogen acceptor in the oxidative process. Thus lactic acid may be reduced to pyruvic acid either by the aerobic process of the reduction of oxygen to water or by the anaerobic processes of the reduction of nitrate to nitrite. Other substances capable of replacing oxygen are: fumaric acid by reduction to succinic; aspartic acid by deamination to fumaric and subsequent reduction; glycerol by reduction to dimethylene glycol, and carbon dioxide by reduction to methane. The ability to use these very various substances as oxidizing agents depends on specific activating enzymes, the possession of one or more of which enables various bacterial species to function as anaerobes.

Strict aerobes, such, for example, as the timothy bacillus, possess none of these enzymes, but have, on the other hand, a very complete battery of mechanisms activating molecular oxygen. In accordance with this observation, it was shown by a series of quantitative experiments that the timothy bacillus oxidizes its substrate as completely as a typical aerobic animal.

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*Regular Meeting, Oct. 30, 1931*

BALDUIN LUCKÉ, *President, in the Chair*

THE ANNUAL GROSS LECTURE: GENERALIZED OSTEITIS FIBROSA AND ITS RELATION TO OSTEOMALACIA AND RICKETS. F. J. LANG, Professor of Pathology and Director of the Pathological Anatomical Institute of the University, Innsbruck, Austria.

Recent views in regard to the nature of osteitis fibrosa were discussed, especially the work of Pommer, who attributes the fibrosis of the bone marrow to the result of localized congestion and irritation and considers the cysts as results of hemorrhages.

The lecturer made extensive studies of the relationship between osteomalacia, rickets and osteitis fibrosa. He considered that parts of the skeleton subjected especially to strain become bent because of insufficient calcification. Owing to the peculiar structure and circulatory system of bone, permanent congestion of blood and lymph vessels result. The congestion and mechanical irritation then lead to osteitis fibrosa. The fibrosis of the bone marrow and formation of bone are secondary to circulatory disturbances that follow functional mechanical trauma in insufficiently calcified and softened bone.

In experimental and clinical studies of rickets, scurvy and osteomalacia, the lecturer regularly found osteitis fibrosa. Localized osteofibrotic changes are also observed in inflammatory processes, as in odontogenetic osteitis fibrosa of the jaw, in the immediate vicinity of metastases to bones, in callus formation and pseudarthrosis, in gout, and near tuberculous and syphilitic lesions of bone. This proves the secondary nature of osteitis fibrosa. In order to establish the basic nature of the disease, it was necessary to use exacting histologic methods showing both calcified and noncalcified bone, by means of many large sections from various bones, seeking the primary changes unobscured by fibrosis. Without such careful methods, incorrect diagnosis of primary osteitis fibrosa will be made.

The peculiar structure of bone and its circulatory system permit only a slight compensatory adaptation to circulatory disturbances. This and the irritation caused by the use of the bone under the given conditions are largely responsible for the origin of osteitis fibrosa. The functional point of view has proved to be of importance in explaining the relation between osteitis fibrosa, osteomalacia and rickets.

## Book Reviews

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**Pathologie und Klinik in Einzeldarstellungen.** Herausgegeben von L. Aschoff, H. Elias, H. Eppinger, C. Sternberg und K. F. Wenckebach. Band III. **Die Lebensvorgänge im normalen Knorpel und seine Wucherung bei Akromegalie.** Von Professor Dr. J. Erdheim, a. ö. Professor an der Universität Wien. Paper. Price, 18 marks. Pp. 160, with 31 illustrations. Berlin: Julius Springer, 1931.

The monograph is divided into four chapters. The first discusses the progressive changes during advancing age in the normal costal cartilage. The mode of origin and the histologic structure of the three cortical layers and of the nucleus of the costal cartilages are described. The degenerative changes appearing with advancing age are detailed, and the influence of these on the changes in the elasticity of the cartilage is coordinated. The healing and restoration that follow degeneration, especially in the nucleus of the cartilage, are also detailed. The chapter ends with a description of the costochondral junction, of the regressive changes occurring at the junctions, of the cement lines, of chondrophyte formation and, finally, the changes appearing in the cartilages during calcification.

The second chapter relates the changes found in the costal cartilages of a woman 71 years of age who suffered from clinical acromegalia for four years, and in whom at autopsy a small tumor was found in the anterior lobe of the pituitary body. The gland weighed 2.35 Gm. Death was due to apoplexy and bronchopneumonia. The bones were normal.

The cartilages, especially the costal, showed what Erdheim designates as the specific changes of acromegalia. This consists of enlargement of the second cortical layer due to hypertrophy and hyperplasia of the cells and an increase of the basophilic ground substance. This enlarged, thick layer appears white in the gross. On the contrary, in normal cartilages the second cortical layer is thin and yellow. The cartilage growth begins at the costochondral junction. Here it is most extensive, and a rosary results. The thickness of the cartilage diminishes as the sternum is approached. Erdheim indicates that in young adults with acromegalia proliferation of the costal cartilages may be much more extensive and much more uniform than in the case being described, and that other cartilages (epiphyseal) may participate in the general growth process.

The third cortical layer and the nucleus do not participate in the hypertrophic and hyperplastic process in advanced life, because they undergo degeneration. In the rib cartilages of patients with acromegalia numerous small and large spaces appear in these zones. The cavities result from resorption by tissue fluids and are most numerous near the costochondral junctions. Many become filled with connective tissue, but they may also contain cartilage débris. On the other hand, the connective tissue contained within these spaces may undergo cartilaginous transformation. Marrow may extend from the bony portion of the rib into the degenerated cartilage nucleus, and free hemorrhages may be present. Of the changes described, the one most stressed is proliferation and hypertrophy of the second cortical layer.

The third chapter deals with acromegalic changes in the joint cartilages. Erdheim also found specific changes, and he distinguishes them from those observed in primary arthritis deformans. They consist of hyperplasia and hypertrophy of the cells of the deeper portion of the articular cartilage. It is similar to the rib in that the proliferating cartilage is at some distance from the perichondrium. These changes are therefore distinguished from primary arthritis deformans, in which the cartilage proliferation begins on the surface. In acromegalia ulceration of the articular cartilage may eventually occur, but it progresses from the deeper

portion toward the surface. In this way the changes again differ from those of primary arthritis deformans, for in this condition the ulceration is from the surface downward. In addition, Erdheim points out that even when the joint cartilage changes of acromegalia are advanced, blood vessels do not penetrate the calcium-free cartilage, while in primary arthritis deformans vessels enter the calcium-free cartilage from the very beginning. The joints both in primary arthritis deformans and in acromegalia have peripheral exostoses.

The fourth chapter deals with the clinical application of the anatomic findings. In a few pages there is summarized much that is known concerning the relation of the pituitary to bone growth, especially in regard to dwarfism. Erdheim points out that a pituitary destruction at a very early age will lead to dwarfism and a delay in closure of the epiphyseal cartilage plates. Pituitary hypersecretion leads to prolonged endochondral ossification. He restates the recent view that the pituitary factor may be more important than the thyroid factor in the pathogenesis of cretinism. He speculates that since the pituitary stimulates cartilage growth, it may affect metabolism (he apparently means sulphur metabolism). He suggests that the surgeon consider the use of anterior pituitary preparations to hasten the union of fractures or in the treatment for delayed union. (The reviewer doubts very much whether the present pituitary preparations are sufficiently satisfactory to test this point. The local factor in delayed union is beyond question.) He advises physiologists and experimental pathologists to investigate the cartilages when studying the tissue changes following pituitary experiments. He suggests that there may be a pituitary factor in chondrodystrophy in which there is arrest of cartilage growth. Erdheim stretches the point when he tries to associate the cartilaginous metastases in the lungs of a patient with acromegalia suffering from an osteochondroma of the femur with pituitary hypersecretion.

The monograph bears the stamp of Professor Erdheim's knowledge of the pathologic anatomy of bone. This is a subject in which he has been continuously interested since his significant work on the relation of the parathyroids to rickets. Figures 2 and 19 show discrepancies between the legends and the markings of the photographs. The monograph also suffers from repetition. The book is recommended for those interested in pituitary disease and in the pathologic anatomy and physiology of bone.

**Anatomie pathologique.** By Maurice Letulle, professeur honoraire a la faculté de médecine de Paris, médecin honoraire de l'hôpital Boucicaut. Avec la collaboration de L. Nattan-Larrier, professeur au collège de France et A. Jacquelin, médecin des hôpitaux de Paris; L. Duclos, ophthalmologiste adjoint de l'hôpital chirurgical Gouin. E.-P. Normand, conservateur du musée d'anatomie pathologique générale Maurice-Letulle (hôpital Boucicaut). Three volumes. Price, 520 francs. Pp. 2,346, with 843 figures. Paris: Masson & Cie, 1931.

This is a posthumous work. The manuscript was completed by Maurice Letulle a few weeks before his death. In response to his expressed wish, it is published without any changes. In the preface by the author's son, Raymond Letulle, he states that the section on the mammary gland has been added because no such section was found among his father's papers. The chapters on the blood and on the visual apparatus were prepared in collaboration with A. Jacquelin and L. Duclos, respectively.

The work comes in three volumes, each containing more than seven hundred pages numbered consecutively, with an alphabetical index and an analytic table of contents at the end of the third volume. In neither the index nor the table is there any mention of the volume in which any particular page occurs, and the pages in each volume are not given on its back, a defect easily remedied, however, by marking the pages in each volume on the back. The binding is not substantial. The type is clear and easy to read, and the pages are pleasant to the eye. The illustrations, 843 in all, all in black and white, mostly drawings, all original, made

and arranged with taste and care, are of high and uniform excellence, in some cases perhaps a little too diagrammatic, but taken all in all of superior instructional value.

In the text as well as in footnotes reference is made frequently to other writers whose names are given, but without mentioning the place and time of publication, thus saving space and labor without much serious loss in view of the extent to which the medical literature now has been indexed in readily available forms. Only a very few American writers are quoted (blastomycosis is not mentioned).

The text is divided into a general part, devoted mainly to inflammatory processes and tumors. Then come sections on the circulatory and respiratory apparatus, the blood and blood-making organs, the digestive organs, the urogenital organs, the endocrine gland, the nervous system and the visual apparatus. The skin, the ear and the locomotive apparatus do not receive separate consideration. The section on the nervous system is incomplete; epidemic poliomyelitis, encephalitis, herpes, syringomyelia, etc., are not discussed.

The discussion is confined strictly to the gross and microscopic morphology of morbid lesions. The descriptions are clear, graphic and orderly. The lesions of syphilis and of tuberculosis are described excellently. The treatment of the lesions of syphilis in descriptive text and by illustrations merits special commendation. The illustrations of buccopharyngolaryngeal sporotrichosis are striking. As would be expected, the fungus of sporotrichosis is named erroneously *Sporotrichum beurmanni* in place of *Sporotrichum schenckii*. Curiously, there are no illustrations of the cells of the blood, and the recent developments in the cytology of leukocytes, endothelial cells and wandering cells are not considered. The reticulo-endothelial system is not considered as such. The tumors in general, and particularly the melanomas, are well described and illustrated.

Letulle's "Anatomie pathologique" is an interesting and valuable book. It gives a good picture of the French teachings in its field, and certainly merits a place in medical libraries.

**Hieronymi Fracastorii de Contagione, et Contagiosis Morbis et eorum Curatione, Libri III.** Translation and Notes by Wilmer Cave Wright, Ph.D., Professor of Greek in Bryn Mawr College. Cloth. Price, \$4.50. Pp. 356. New York: G. P. Putnam's Sons, 1930.

Fracastorius, 1478-1553, is known best as the author of the famous poem "Syphilis," which was published in its final form in 1530 and in which he treats of the disease that since has borne the name he gave the poem. Numerous editions in Latin and several translations of the poem have appeared. A far more important work by Fracastorius is his prose treatise "De Contagione, et Contagione Morbis et eorum Curatione," published in 1546 and now for the first time translated into English. The translation is published as the second volume of the History of Medicine Series issued under the sponsorship of the Library of the New York Academy of Medicine.

In this book infectious diseases are considered for the first time under three groupings, namely, infections by direct contact, infections carried by intermediate agents, or formites as Fracastorius named them, and infections that come through the air from a distance. The discussion is carried on in terms that suggest a truly remarkable prevision of the discoveries in microbiology more than three hundred years later. Typhus is recognized as a distinct disease, and other diseases (syphilis, tuberculosis of the lungs, plague, rabies, exanthems) are considered. The translation and notes are the product of mature and thorough scholarship. The introduction gives a most interesting account of the life of Fracastorius, and the bibliography lists the important editions and translations of his books, as well as biographies and pertinent general works. A great medical classic has received a worthy English setting.



## Books Received

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CHEMICAL METHODS IN CLINICAL MEDICINE. By G. A. Harrison, B.A., M.D., B.Ch., M.R.C.S., L.R.C.P., Reader in Chemical Pathology in the University of London; Reader and Lecturer on Chemical Pathology in St. Bartholomew's Medical College. Cloth. Price, \$5.25. Pp. 534. New York: The Macmillan Company, 1930.

VERHANDLUNGEN DER DEUTSCHEN PATHOLOGISCHEN GESELLSCHAFT. Im Auftrage des Vorstandes herausgegeben von dem derzeitigen Schriftführer G. Schmorl in Dresden. Sechszwanzigste Tagung gehalten in München am 9.-11. April, 1931. Pp. 423. Mit 193 Abbildungen im Text und 11 Tafeln. Jena: Gustav Fischer, 1931.

THE QUANTITATIVE ESTIMATION OF VITAMIN D BY RADIOGRAPHY. By R. B. Bourdillon, H. M. Bruce, C. Fischmann and T. A. Webster. Medical Research Council, Special Report Series, No. 158. Price, 6 pence, net. Pp. 46. London: His Majesty's Stationery Office, 1931.

THE INFLUENCE OF DIET ON CARIES IN CHILDREN'S TEETH (INTERIM REPORT). By the Committee upon Dental Disease. Medical Research Council, Special Report Series, No. 159. Price, 6 pence, net. Pp. 19. London: His Majesty's Stationery Office, 1931.

SURGICAL PATHOLOGY OF THE SKIN, FASCIA, MUSCLES, TENDONS, BLOOD AND LYMPH VESSELS. By Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kansas, and Professor of Surgery, University of Kansas. Price, \$5. Pp. 301, with 260 illustrations. Philadelphia: J. B. Lippincott Company, 1931.

A SYSTEM OF BACTERIOLOGY IN RELATION TO MEDICINE. Volume 9. Medical Research Council. Price, per volume, 1 pound, 1 shilling, net. London: His Majesty's Stationery Office, 1931. (It may be obtained from the British Library of Information, 5 East Forty-Fifth Street, New York.)

EIN BEWEGTES GELEHRTENLEBEN. ERINNERUNGEN UND ERLEBNISSE, KÄMPFE UND GEDANKEN. Von Otto Lubarsch. Pp. 606. Berlin: Julius Springer, 1931.

HIERONYMUS FRACASTORIUS. CONTAGION, CONTAGIOUS DISEASES AND THEIR TREATMENT. Translation and Notes by Wilmer Cave Wright, Ph.D., Professor of Greek in Bryn Mawr College. Pp. 356. New York: G. P. Putnam's Sons, 1930.

GRUNDRISS DER ENTWICKLUNG DES MENSCHEN. Von Dr. Alfred Fischel, o. Professor der Embryologie und Vorstand des Embryologischen Institutes der Wiener Universität. Paper. Price, 11 marks. Pp. 141, with 117 illustrations. Berlin: Julius Springer, 1931.

REPORT OF COMMITTEE ON STANDARD PRACTICES IN THE PROBLEM OF COMPENSATION OF OCCUPATIONAL DISEASES OF THE INDUSTRIAL HYGIENE SECTION, AMERICAN PUBLIC HEALTH ASSOCIATION. By Henry H. Kessler, M.D. (Chairman), Bernard S. Coleman, Emery R. Hayhurst, M.D., George M. Price, M.D., May R. Mayers, M.D., Elizabeth B. Bricker, M.D., R. R. Sayers, M.D., and Eleanor Rantoul. Paper. Price, \$1.50. Pp. 124. New York: American Public Health Association, 1931.

LES EXTRAITS PANCRÉATIQUES DÉINSULINÉS EN THÉRAPEUTIQUE. Par R. Giroux et N. Kisthinos, assistants à la clinique thérapeutique de l'Hôpital de la Pitié. Préface du Professeur H. Vaquez. Paper. Price, 16 francs. Pp. 126, with 9 illustrations. Paris: Masson & Cie, 1931.

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